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Spectrophotometric and chromatographic methods for the determination of a binary mixture of sodium cromoglicate and xylometazoline hydrochloride

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ABSTRACT

Simple, sensitive and precise spectrophotometric and chromatographic methods were developed for simultaneous determination of Sodium cromoglicate (SCG) and Xylometazolinehydrochloride(XYLO) in their pure form and nasal solution. By applying the spectrophotometric methods, (SCG) was determined by direct spectrophotometry through measuring its zero-order (D_0) absorption spectra at 325 nm; while (XYLO) was determined using two methods. The first method utilized the first derivative spectra $D^1$of (XYLO) after prior separation of SCG from medium using 0.1 N HCl. The second method depends on derivative compensation ratio technique depending on the mean ratio of D^1 peak amplitudes of SCG ($D_{231.4}$ / $D_{309.4}$). The chromatographic method utilized aZorbax SB-C_{18} column and a mobile phase of acetonitrile: 10 mM phosphate buffer (pH 4): triethylamine, in ratio (80: 19.9: 0.1 v/v/v), delivered at flow rate of 1.2 mL/minand the detections were done at 220 nm. The proposed methods were validated in compliance with the ICH guidelines and were successfully applied for the analysis of (SCG) and (XYLO) in the laboratory prepared mixtures and combined pharmaceutical dosage form.

KEYWORDS

Sodium cromoglicate;
Xylometazoline hydrochloride;
Derivative spectrophotometry;
Derivative compensation ratio technique;
Chromatographic.

INTRODUCTION

Sodium cromoglicate (SCG) [disodium 4,4′-dioxo-5,5′-(2-hydroxytrimethylenedioxy) di(4*H*-chromene-2-carboxylate)] is a mast cell stabilizer that inhibits the release of histamine and other inflammatory mediators from sensitized mast cells. Xylometazoline hydrochloride (XYLO) 2-[[4-(1,1-Dimethylethyl)-2,6–dimethylphenyl]methyl]-4,5–dihydro–1-*H*-imidazole, hydrochloride, is a direct-acting sympathomimetic used as a vasoconstrictor for congestion of mucous membranes. Both drugs are formulated together in the form of nasal solution for treatment of rhinitis[1]. The chemical structures of both drugs[2] were shown in Figure 1.

A survey of the literature revealed the methods

reported for the determination of SCG such as UV spectrophotometry[3,4], HPLC[5,6], TLC[7] and capillary electrophoresis[8]; and for XYLO such as UV spectrophotometry[9,10], HPLC[11,12] and TLC[13].

No methods have been reported for the analysis of the binary mixture of these two drugs. The analysis of this binary mixture represented a challenge through the complete overlapped spectra of the major component SCG and minor component XYLO; where the ratio of the drugs in the dosage forms SCG: XYLO (80:1) and the huge difference in absorptivities values of both drugs. The aim of this work is to develop simple, sensitive and precise spectrophotometric and chromatographic methods for the determination of SCG and XYLO in their binary mixture.

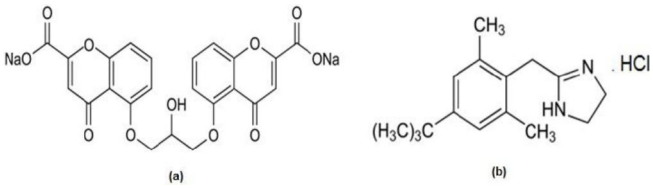

Figure 1 : Chemical structures of (a) Sodium cromoglicate (SCG) and (b) Xylometazoline hydrochloride (XYLO)

Theory of the Derivative compensation ratio technique

This method[14-16] is used when the absorption curves of the 2 components (x) and (y) overlap to a large extent or when analysis of a minor component in the presence of a major component represents a problem, the balance point or the graphical method can be adopted. Under these conditions the absorption spectrum of mixture of two drugs (x) and (y) assumed to possess gross curve which has no characteristics of either of the pure compounds.

Balance point

The compensation method involves recording derivative spectra of the difference absorption spectra of [mixture (m) – reference (r)] where ($m = A_x + A_y$) and ($r = A_x$) using different concentration of pure drug (x) above and below that expected in the mixture solution in the reference cell C_x. The D^1 amplitude ratios (wavelength D_a/wavelength D_b) are determined in chosen order (D). The D^1amplitude ratios of difference spectra are compared with the standard D^1amplitude ratio of pure (C_y) against a blank solution. The amplitude ratios characteristics of the mixture gradually approach that of pure drug (y) as C_x increases and finally coincide with amplitude ratio of pure drug (y) at the balance point, for which C_x in the mixture equal to C_x in the reference cell.

Graphical method

It consists of plotting the above mentioned D^1 amplitude ratios of the mixture against the concentration of the drug (x) in the reference cell where a line with very slight curvature is obtained. The concentration of the drug (x) is calculated from the graph as it is the concentration corresponding to the amplitude ratio of the mixture which is equal to the standard amplitude ratio of the pure component (y) previously determined as the mean amplitude ratio (D_a / D_b) obtained from derivative spectra of pure (y) at different concentration.

Modified graphical method via regression equation

This is a new modification, the regression equation represent the Graphical method was computed, the concentration of (x) could be determined after substitution in it using the average of standard amplitude ratio.

MATERIALS AND METHODS

Apparatus and software

Shimadzu - UV 1800 double beam UV-Visible spectrophotometer (Japan) with matched 1 cm quartz cells at 200-800 nm range was used for all absorbance measurements. Spectra were automatically obtained by Shimadzu UV-Probe 2.32 system software.

Agilent 1200 series chromatographic system equipped with quaternary pump, microvacuum degasser, thermostatted column compartment and variable wavelength UV–VIS detector was used. Sample injections were made through an Agilent 1200 series autosampler. Data collection and processing were performed using Agilent ChemStation software, version A.10.01. Column Agilent Zorbax SB-C_{18} (150mm×4.6 mm, 5 µm particle size i.d.) was from (Agilent Technologies, Polo Alto, CA, USA). A "Jenway 3505" pH-meter (Jenway, UK), equipped with combined glass electrode was used for pH adjustment.

Chemicals and reagents

Pure samples

Sodium cromoglicate and Xylometazoline

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hydrochloride were kindly supplied by Sigma Pharmaceutical Industries Limited; Al-Monofeya, Egypt. Their purity was found to be 100.80 ±0.74 and 100.18 ±0.47 for SCG and XYLO respectively by the official methods.[3]

Market sample

Nasotal Compound ® nasal drop, labeled to contain 2% SCG and 0.025 % XYLO per 100 mL, (Batch no: 094244) was manufactured by Amoun Pharmaceutical Co, Egypt.

Solvents

Spectroscopic analytical grade of methanol (S.d.fine-chem limited- Mumbai), 33% HCl, 85% o-phosphoric acid (Biochem-Egypt) and distilled water were used.

Standard solutions

Stock solutions of SCG and XYLO of concentration 2 mg/mL were prepared in a solvent mixture of water: methanol (70:30 v/v) at 4 °C. Working solutions were freshly prepared by dilution from the stock solutions with solvent mixture to get 50 µg/mL and 100µg/mL of SCG and XYLO respectively.

Procedure for spectrophotometric methods

Construction of calibration curves

For SCG

Different aliquots were transferred from its working solution (50 µg/mL) into a series of 10 mL volumetric flasks, and the volume was completed with solvent mixture to make up solutions in the range of (7.5-50 µg/mL). The absorption spectra were measured for the prepared solutions at (200-400 nm). Calibration curves were constructed relating the D_0 absorbance at 325 nm to the corresponding concentration against solvent mixture as a blank and the regression equation was computed.

For XYLO

Different aliquots were transferred from its working solution (100µg/mL) into a series of 10 mL volumetric flasks, complete to 3 mL with solvent mixture, then complete to volume with 0.1 N HCl to form solutions in a concentration range of (4 -22µg/mL). The absorption spectra were measured for the prepared solutions at (200-400 nm). The first derivative D^1 was calculated for the obtained spectra ($\Delta\lambda$ = 8, scaling factor 50). Calibration curves were constructed relating the D^1 absorbance at 230.4 nm to the corresponding concentration and the regression equation was computed.

Derivative compensation ratio technique

The first derivative D^1 was calculated for the obtained spectra ($\Delta\lambda$ =8, scaling factor 50). The amplitude was measured at 231.4 nm and 309.4 nm. The mean ratio of peak amplitudes of the first derivative D^1 of SCG was calculated at 231.4 nm and 309.4 nm ($D_{231.4}$ / $D_{309.4}$).

A mixture solution containing (40 µg/mL) of SCG and (2.5 µg/mL) of XYLO was prepared from the working solutions. The mixture solution was transferred to the sample cell. A series of solutions was prepared containing various concentrations of pure XYLO above and below that present in mixture (0 – 3 µg/mL), and placed in the reference cell successively. The spectra of the mixture solution against each reference cell solution were recorded at (200- 400 nm). SCG was determined using its D_0 absorbance at 325 nm. The first derivative $D^1$was recorded and the derivative amplitude ratio of the mixture (in sample cell) was calculated and plotted against the concentration of XYLO (in reference cell). A straight line is obtained and its regression equation is calculated. The concentration of XYLO is calculated from the regression equation as its concentration will be corresponding to mean standard amplitude ratio of pure SCG previously determined ($D_{231.4}$ / $D_{309.4}$).

Procedure for chromatographic method

Chromatographic conditions

RP-HPLC was carried out at ambient temperature on Zorbax SB-C_{18}column. The mobile phase consisted of acetonitrile: 10 mM phosphate buffer (pH 4): triethylamine,in ratio (80: 19.9: 0.1 v/v/v). The mobile phases was filtered using 0.45 ìm Millipore membrane filter (Billerica, MA) and was delivered at flow rate of 1.2 mL/min. The injection volumes were 20 ìL and the detections were done at 220 nm. The analysis was performed at ambient temperature (25 °C).

System suitability

Twenty microliters of the working solutions were injected and applied to the chromatographic conditions.

The system suitability parameters, retention time, tailing factor, theoretical plate count (N), height of theoretical plate (HETP), separation of peaks (resolution) and column capacity were calculated and compared to reference value [99, 100].

Construction of calibration curves for chromatographic method

Different aliquot volumes were separately transferred from the SCG stock solution (1 mg/mL) and XYLO working solution (100µg/mL) into two sets of 10 mL measuring flasks, diluted to the volume with the mobile phase to form solutions in the concentration range of (100– 900 µg/mL) of SCG and (10– 80 µg/mL) of XYLO. Twenty microliters of these solutions were injected in triplicate into the HPLC system. The chromatographic conditions were applied and the chromatograms were recorded. The calibration curve of each drug was constructed by plotting the relative peak area [the peak area found to that of an external standard of the same drug (100µg/mL of SCG and 10µg/mL XYLO) against the corresponding concentration at 220 nm. The corresponding regression equations were calculated. The calibration curves were made from the average of three experiments.

Analysis of laboratory prepared mixtures

Using D^1 method of XYLO coupled with prior separation of SCG in 0.1 N HCl

Into a series of 10 mL volumetric flasks (A), accurate aliquots of SCG and XYLO were transferred separately from their working solutionsto prepare mixtures containing different ratios of the two drugs in the concentration range of (7.5 – 50 µg/mL) of SCG and (0.5 –2 µg/mL) of XYLO and then complete to volume using solvent mixture. Into another series of 10 mL volumetric flasks (B), accurate aliquots were transferred separately from SCG standard solution (2 mg/mL) and XYLO working solution (100 µg/mL) to prepare mixtures containing different ratios of the two drugs (160 – 480 µg/mL) of SCG and (4 – 22 µg/mL) of XYLO, complete to 3 mL using solvent mixture; then 5 mL of 0.1 N HCl was added to precipitate SCG and left for 10 minutes. The solution was filtered using double filter paper and then volume was completed using 0.1 N HCl. Record and store the spectra of the prepared solutions of both series from 200 to 400 nm. Proceed as detailed under linearity for both drugs where SCG was determined from series (A) using its D_0 absorbance at 325 nm, while XYLO was determined from series (B) using its D^1 amplitude at 230.4 nm. The concentrations of both drugs were calculated through substitution in their corresponding regression equation.

Using chromatographic method

Different aliquot volumes of SCG and XYLO were accurately transferred from their working solutions and mixed to prepare solutions of different ratios in the concentration range of (100– 900 µg/mL) of SCG and (10– 80 µg/mL) of XYLO. The chromatographic conditions were adopted for each laboratoryprepared mixture and the concentrations for each drug were calculated from the regression equations. Each concentration was conducted from the average of three experiments.

Application to pharmaceutical preparation

Five milliliter of Nasotal Compound ® nasal drops were transferred into 25 mL volumetric flask, filtered if necessary through 0.45 µm Millipore syringe membrane filter and complete to volume with solvent mixtureto obtain a solution (a) concentration of 4 mg/mLof SCG and 50 µg/mLof XYLO. For the determination of SCG, further dilution was done using solvent mixture to obtain a solution of a concentration of 40 µg/mLof SCG and 0.5 µg/mLof XYLO. The claimed concentration of SCG was determined using its D_0 absorbance at 325 nm.The determination of XYLO was performed using the two following procedures.

Using derivative compensation ratio technique

One milliliter was accurately transferred from solution (a) into 100 mL volumetric flask, then the solution was spiked with 200µg of XYLO from its working solution and complete to volume with solvent mixtureto obtain a solution of concentration of 40 µg/mLof SCG and 2.5 µg/mLof XYLO. And then proceed as described under the Derivative compensation ratio technique. The claimed concentration of XYLO in the solution was calculated after subtraction of the added concentration (XYLO solution 2 µg/mLanalyzed by using the same procedure).

Using D^1 method of XYLO coupled with prior separation of SCG in 0.1 N HCl

One milliliter was accurately transferred from

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solution (a) into 10 mL volumetric flask, the volume was completed to 3 mL with solvent mixture, then 5 mL of 0.1 N HCl was added and left for 10 minutes. The solution of was then filtered using double filter paper, then the solution was completed to the volume using 0.1 N HCl,to obtain a final concentration of 400µg/mLof SCG and 5 µg/mLof XYLO. Then XYLO was determined using its D^1 amplitude at 230.4 nm.

Proceed as detailed under linearity for both drugs and calculate the concentration of each drug using its corresponding regression equation. When carrying out the standard addition technique, different known concentrations of pure standard SCG and XYLO were added to the pharmaceutical dosage form before proceeding in the previously mentioned methods .

Using chromatographic method

An appropriate dilution was made from solution (a) with the mobile phase to prepare the working solution to obtain two solutions: the first solution (x) of a concentration of 400µg/mL of SCG and 5µg/mL of XYLO; and the second solution (y) of a concentration of 800µg/mL of SCG and 10µg/mL of XYLO. Twenty microliters of the solution was applied and the chromatographic conditions were applied. Solution (x) was used for the determination of SCG, while Solution (y) was used for the determination of XYLO.

Proceed as detailed under linearity for both drugs and calculate the concentration of each drug using its corresponding regression equation. When carrying out the standard addition technique, different known concentrations of pure standard SCG and XYLO were added to the pharmaceutical dosage form before proceeding in the previously mentioned methods.

RESULTS AND DISCUSSION

Spectrophotometric methods

The major problem was raised upon the analysis of this binary mixture which was the complete overlapped spectra of the major component SCG and minor component XYLO; where the ratio of the drugs in the dosage forms SCG: XYLO (80:1). To facilitate the determination of the minor component (XYLO), there is a need to increase its concentration in the mixture using standard addition technique [69, 182]. This is done adding fixed amounts of standard XYLO to each experiment then subtract its concentration before calculating the claimed concentration of the drug. The UV absorption spectra (D_0) of SCG and XYLO are shown in Figure 2a, where SCG could be easily detected by direct spectrophotometry; But XYLO couldn't be resolved by derivative spectrophotometry or other manipulating techniques.

The SCG concentrations were calculated using the regression equations representing the linear relationship between the zero order spectra D_0 at 325nm versus the corresponding concentrationsin the range (7.5-50 µg/mL), while the two following methods were described for the analysis of XYLO in this binary mixture.

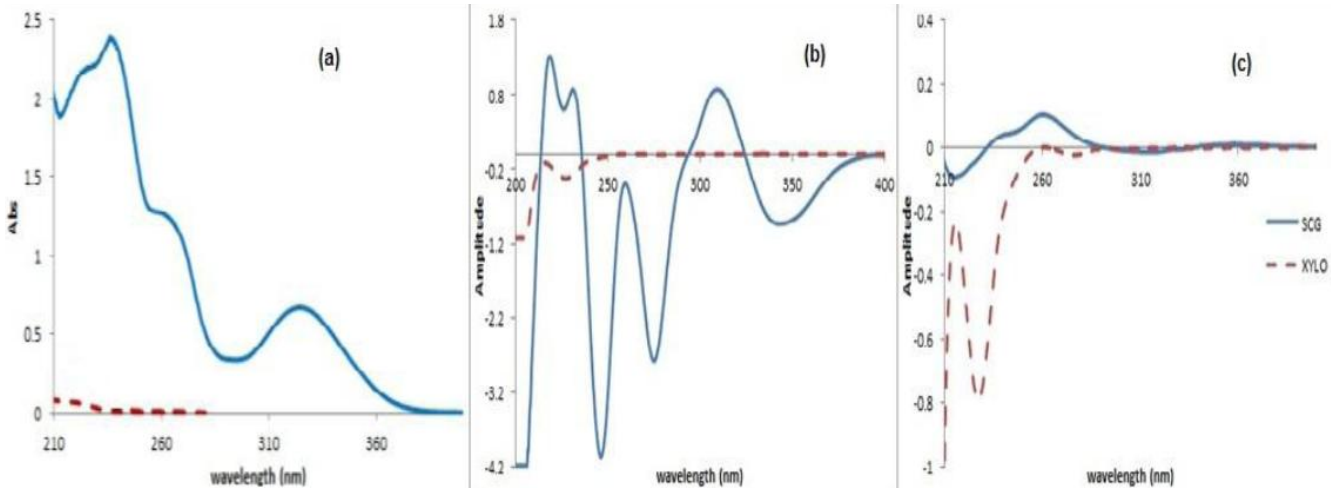

Figure 2 : Chemical structures of (a) 40 µg/mL of SCG and 2 µg/mL in water: methanol (70:30) (b) D^1 spectra of 40 µg/mL of each of SCG and 2.5µg/mL XYLO in water: methanol (70:30), (b) 400 µg/mL of SCG and 4µg/mL XYLO in 0.1 N HCl.

Derivative compensation ratio technique

The first method depends on derivative compensation ratio technique. Figure 2b shows D^1 of SCG and XYLO. The mean ratio of D^1 peak amplitudes of SCG ($D_{231.4}$ / $D_{309.4}$) was calculated to be 0.9663. A mixture solution was prepared assembling the concentration of both drugs in the pharmaceutical dosage form (40µg/mLof SCG and 2.5 µg/mLof XYLO). The solution was placed in the sample cell. Different concentrations of pure XYLO (0 – 3 µg/mL) are placed in the reference cell successively. The spectra of the mixture solution against each reference cell and the first derivative D^1 were recorded. The straight line obtained, Figure 3 and its regression equation was found to be:

P = 0.1481 C + 0.5975 r = 0.9995

Where P is the amplitude ratio of the mixture ($D_{231.4}$ / $D_{309.4}$) against different reference cells, C is the concentration of pure XYLO in reference cell; r is the correlation coefficient.

The concentration of XYLO is calculated by substituting in the regression equation with the mean standard amplitude ratio of pure SCG previously determined ($D_{231.4}$ / $D_{309.4}$ = 0.9663).

Derivative spectrophotometry with prior physical separation

The second method was described for the analysis of XYLO in this binary mixture depending on the prior separation of SCG by precipitation using acidic medium (0.1 N HCl). The mixtures containing SCG and XYLO were treated by 5 mL of 0.1 N HCl, where SCG will be precipitated after 10 minutes and removed from the medium by filtration. XYLO was then determined by through the first derivative spectra D^1 showing zero crossing with SCG spectrum in the concentration range of (4 -22 µg/mL), Figure 2c. There is no need to add spiked XYLO to the samples using this method, as the major content of SCG will be eliminated from the medium. Different types of acids and different strengths were checked, where 5 mL of 0.1 NHCl showed satisfactory results for both SCG and XYLO, as SCG (partially precipitated) and XYLO were stable in this medium for more than 2 hours. The solutions were left in contact with 0.1 N HCl for 10 minutes to ensure maximum precipitation where no spectral changes occurred.

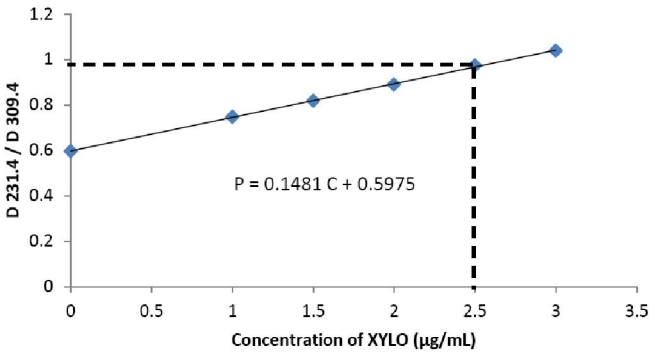

Figure 3 : Graphical representation of the peak amplitude ratio ($D_{231.4}$ / $D_{309.4}$) of SCG and XYLO mixture in sample cell versus concentration of XYLO in reference cell.

Chromatographicmethod

The developed RP-HPLC method utilized C_{18} column and acetonitrile: 10 mM phosphate buffer (pH 4): triethylamine,in ratio (80: 19.9: 0.1 v/v/v) as a mobile phase. The run time per sample was just 3 minutes. To optimize the HPLC method, it was necessary to test the effect of different variables. In order to separate the two drugs from each other, Two types of stationary phases were tried (Zorbax C_8 and Zorbax SB-C_{18} columns), but the later showed better resolution. Several ratios of buffer and acetonitrile were checked. Increasing the ratio of acetonitrile caused broadening of SCG peak, and decreasing its ratio caused overlap of the two peaks. Different types and pH was tried for the buffer which led to unsuccessful separation and broad peaks. Methanol and distilled water wasn't successful for the separation of the two peaks. Different flow rates (0.5-1.2 mL/min) were tested; the flow rate 1.2 mL/min gave the best compromise for separation and peak symmetry. SCG and XYLO were separated at retention time 2.630 and 1.263 respectively, Figure 4. Solutions of (100 µg/mL) of SCG and (10 µg/mL) of XYLO were used as external standards. The detection was carried out at 220 nm to increase sensitivity of XYLO. System suitability parameters for the mixture were calculated and compared to reference value [153]as shown in TABLE 1.

The corresponding concentration ranges, calibration equations and other statistical parameters for the proposed methods were listed in TABLE 2. The proposed procedures were also applied for the determination of SCG and XYLO in Nasotal Compound ® nasal drops. The validity of the proposed procedures is further assessed by applying the standard

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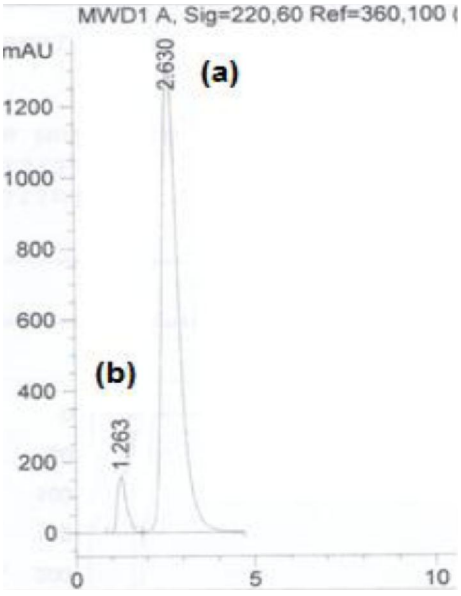

Figure 4 : RP-HPLC chromatogram of (a) 800 µg/mL SCG and (b)10 µg/mL XYLO using C_{18} column and mobile phase of acetonitrile: 10 mM phosphate buffer (pH 4): triethylamine, in ratio (80: 19.9: 0.1 v/v/v), flow rate of 1.2 mL/min at 220 nm.

TABLE 1 : Parameters Required for System Suitability of HPLC method.

Parameter	SCG	XYLO	Reference value[99, 100]
t_R (Relative retention time)	2.630	1.263	
K' (capacity factor)	2	5.26	1-10 acceptable
α (separation factor)	**2.63**		**> 1**
N (Column efficiency)	5109	4667	N > 2000 Increases with efficiency of the separation
HETP (Height equivalent to theoretical plates)	0.002	0.003	The smaller the value, the higher the column efficiency
T (tailing factor)	1.56	0.98	T ≤ 2 T=1 for symmetric peak
Rs (Experimental Resolution)	**2.36**		**Rs> 2**

addition technique. The results obtained are shown in TABLE 3. The specificity of the proposed procedures

TABLE 2 : Assay parameters and methods validation sheet obtained by applying the proposed methods.

Parameters Methods	UV absorbance	First derivative D^1	Derivative compensation ratio	RP-HPLC method	
	SCG	XYLO	SCG	SCG	XYLO
Wavelength (in nm)	325	230.4	($D_{231.4}$ / $D_{309.4}$)	220	
Calibration range [a]	7.5 -50	4 - 22		100 - 900	10 - 80
Regression parameters:					
Slope	0.0167	0.3000	0.1481	0.0097	0.0190
Intercept	0.0011	0.0029	0.5975	0.0112	0.8068
Correlation coefficient (r)	0.9999	0.9999	0.9995	0.9999	0.9998
Mean	100.23	100.23	100.00	100.02	100.06
± SD	± 0.57	± 0.77	± 0.65	± 0.98	± 1.00
RSD	0.57	0.77	0.65	0.98	1.00
Accuracy (mean ± SD)	100.12 ± 0.10	99.95 ±1.11		100.07 ±0.65	99.82 ±0.74
Precision:					
Repeatability [b]	0.50	0.54	0.32	0.76	0.45
Intermediate precision[b]	0.40	0.72	0.43	0.92	0.84
LOD	0.189	0.229		8.69	1.41
LOQ	0.574	0.695		23.33	4.28
Robustness [c]	1.13	0.37	0.41	0.69	0.76

[a]Calibration points: n=8; Concentration in µg/mL.;[b] Average of three experiments; [c] RSD of SCG and XYLO (n=9); LOD: Limit of detection, LOQ: Limit of quantitation; in µg/mL.

was assessed by the analysis of laboratory prepared mixtures containing different ratios of the two drugs, where satisfactory results were obtained as shown in TABLE 4.

TABLE 3 : Application of standard addition technique to the analysis of SCG and XYLO in Nasotal compound ® nasal drops by applying the proposed methods.

Nasotalcompound ® nasal drops (Batch no: 094244)	Claimed (µg/mL)	Found[a] (µg/mL)	Found %	Pure added[b] (µg/mL)	Recovery%
Spectrophotometric methods					
				8	100.43
SCG	40	40.62	101.56 ± 0.29	9	100.19
				10	100.83
Mean ± SD					100.48 ± 0.32
XYLO [c]	0.5	0.49	98.02 ± 0.38		
Mean ± SD					
				5	100.11
XYLO [d]	5	4.94	98.80 ± 0.57	6	99.74
				7	99.34
Mean ±SD					99.73 ± 0.39
Chromatographic method					
				100	100.12
SCG	400	406.12	101.53 ± 0.83	150	99.75
				200	99.32
Mean ±SD					99.73 ± 0.40
				10	100.64
XYLO	10	9.96	98.60 ± 0.42	20	99.65
				30	99.91
Mean ±SD					100.07 ± 0.51

[a]Average of six experiments; [b]Average of three experiments; [c]Calculated by derivative compensation ratio technique (after subtraction of spiked XYLO concentration 2 µg/mL); [d]Calculated by D^1 spectrophotometry after separation of SCG in 0.1 N HCl.

TABLE 4 : Determination of SCG and XYLO in laboratory prepared mixtures by the proposed methods.

Spectrophotometric methods						RP-HPLC method				
Ratio[a]	SCG Set (A)		Ratio[a]	XYLO Set (B)		Ratio[a]	SCG		XYLO	
	Taken[b]	Recovery %		Taken[b]	Recovery %		Taken[b]	Recovery %	Taken[b]	Recovery %
16:2	16	100.76	160:20	20	100.12	10:1	200	100.21	20	99.94
24:1	24	101.5	240:10	10	99.75	20:1	400	99.87	20	101.21
32:1	32	100.16	320:10	10	100.32	30:1	300	101.21	10	100.76
48:0.5	48	99.98	480:5	4	101.13	40:1	400	100.65	10	101.23
40:0.5[c]	40	100.34	400:5[c]	5	99.51	80:1[c]	800	100.43	10	99.76
Mean ± SD		100.55 ± 0.61			100.17 ±0.62	Mean ± SD		100.47 ± 0.50		100.58 ± 0.70

[a]Ratio SCG:XYLO; [b]in µg/mL.; [c]Ratio equivalent to that present in Nasotal Compound® nasal drops; Series A: SCG determined using D_0 at 325 nm.; Series B: XYLO was determined using D^1 after precipitation of SCG in 0.1 N HCl.

METHODS VALIDATION

Method validation was performed according to ICH guidelines[54] for the proposed methods including system suitability, linearity and range, accuracy, specificity, precision, LOD / LOQ and Robustness, TABLE 1 and 2. All the results obtained were within

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acceptable range.

CONCLUSION

The major problem was raised upon the analysis of this binary mixture which was the complete overlapped spectra of the major component SCG and minor component XYLO; where the ratio of the drugs in the dosage forms SCG: XYLO (80:1). From the previous discussion, it could be concluded that the advantages of the spectrophotometric methods that they were able to analyze this complex mixture, while the other manipulatingspectrophotometric and chemometric methods gave bad recovery results. The main disadvantage of the spectrophotometric methods that the first one is performed on two steps; the analysis of SCG followed by its separation by precipitation in acidic medium, then the analysis of XYLO is performed. While the second method required several runs using different concentrations of XYLO in the reference cell. The chromatographic method appliedwas found to be simple, accurate and selective quantitative analysis of the binary mixture in bulk powder, laboratory-prepared mixtures and nasal solution. The advantage of this method was that the analysis was performed in a single step. The disadvantage of RP-HPLC method was that it requires more time as the column requires pre saturation; the high cost of HPLC solvents and the and preparation of buffer. The good recoveries obtained in all cases, proved that the proposed methods could be applied efficiently for determination of this binary mixture with quite satisfactory precision and could be easily applied in quality control laboratory for its analysis.

REFERENCES

[1] S.C.Sweetman; Martindale 36: The Complete Drug Reference, Pharmaceutical Press, **(2009)**.

[2] British Pharmacopoeia; The Stationery Office on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA)-© Crown Copyright **(2009)**.

[3] J.Tillman, D.Whymark; Analyst, **96**, 1147, 689 **(1971)**.

[4] S.T.Hassib, A.A.El-Zaher, M.A.Fouad; Drug Test.Anal., **3(5)**, 306 **(2011)**.

[5] M.S.Ali, S.Rafiuddin, D.A.Al-Jawi, Y.Al-Hetari, M.U.H.Ghori, A.R.Khatri; J.Sep.Sci., **31(9)**, 1645 **(2008)**.

[6] M.Ozoux, J.Girault, J.Malgouyat, O.Pasquier; J.Chromatogr., B, **765(2)**, 179 **(2001)**.

[7] V.Kocić-Pesić, D.Radulovic, D.Pećanac, L.Zivanović; .Farmaco, **47(12)**, 1563 **(1992)**.

[8] A.Helle, S.Hirsjärvi, L.Peltonen, J.Hirvonen, S.K.Wiedmer; J.Chromatogr., A, **1178(1)**, 248 **(2008)**.

[9] S.Khalil; Microchimica acta, **130(3)**, 181 **(1999)**.

[10] Y.Anjaneyulu, M.R.P.Reddy, C.S.Kavipurapu; Analyst, **111**, 1167 **(1986)**.

[11] Z.Milojevic, D.Agbaba, S.Eric, D.Boberic-Borojevic, P.Ristic, M.Solujic; J.Chromatogr., A, **949**, 79 **(2002)**.

[12] Y.Chen, G.Z.Yu; Yaowu Fenxi Zazhi, **30(1)** 142 **(2010)**.

[13] D.Agbaba, Z.Milojevic, S.Eric, M.Aleksic, G.Markovic, M.Solujic; J.Plan.Chrom. - M.TLC, **14(5)**, 322 **(2001)**.

[14] A.A.M.Wahbi, F.A.El-Yazbi, M.H.Barary, S.M.Sabri; Analyst, **117(4)**, 785 **(1992)**.

[15] N.Erk; J.Pharm.Biomed.Anal., **24,** 603 **(2001)**.

[16] A.A.M.Wahbi, O.Abdel-Razak, A.A.Gazy, H.Mahgoub, M.S.Moneeb; J.Pharm.Biomed.Anal., **30**, 1133 **(2002)**.