Supplementary material

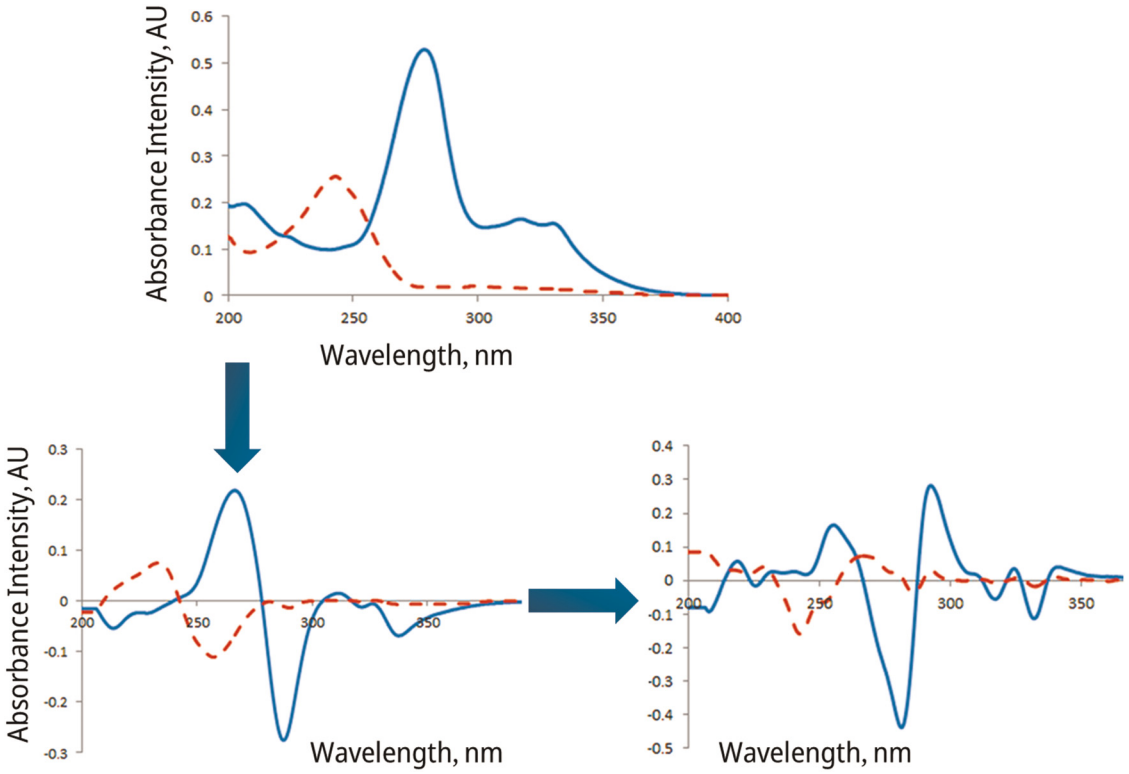

Figure S1: (a–c): Zero order UV-spectra, D0, (a). first derivative spectra, D1, (b). Second derivative spectra, D2, (c) of 5 µg/mL of each CIP ((____and HYD (– – – –).

Analytical Greenness report sheet

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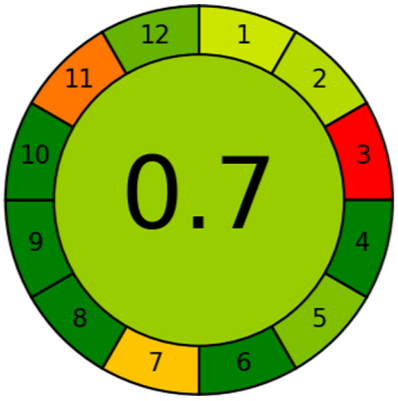

Criteria	Score	Weight
1. Direct analytical techniques should be applied to avoid sample treatment.	0.6	2
2. Minimal sample size and minimal number of samples are goals.	0.65	2
3. If possible, measurements should be performed in situ.	0.0	2
4. Integration of analytical processes and operations saves energy and reduces the use of reagents.	1.0	2
5. Automated and miniaturized methods should be selected.	0.75	2
6. Derivatization should be avoided.	1.0	2
7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.	0.39	2
8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	1.0	2
9. The use of energy should be minimized.	1.0	2
10. Reagents obtained from renewable sources should be preferred.	1.0	2
11. Toxic reagents should be eliminated or replaced.	0.23	2
12. Operator's safety should be increased.	0.8	2

Figure S2: The Analytical Greenness report sheet for the UV-spectrophotometric method.

Analytical Greenness report sheet

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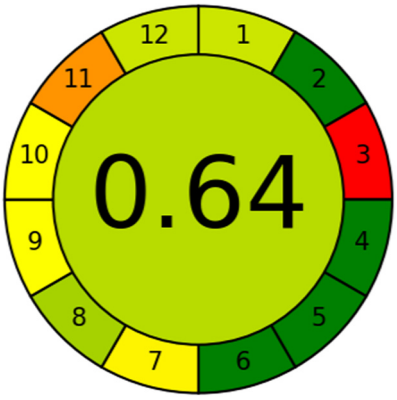

Criteria	Score	Weight
1. Direct analytical techniques should be applied to avoid sample treatment.	0.6	2
2. Minimal sample size and minimal number of samples are goals.	1.0	2
3. If possible, measurements should be performed in situ.	0.0	2
4. Integration of analytical processes and operations saves energy and reduces the use of reagents.	1.0	2
5. Automated and miniaturized methods should be selected.	1.0	2
6. Derivatization should be avoided.	1.0	2
7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.	0.48	2
8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	0.68	2
9. The use of energy should be minimized.	0.5	2
10. Reagents obtained from renewable sources should be preferred.	0.5	2
11. Toxic reagents should be eliminated or replaced.	0.29	2
12. Operator's safety should be increased.	0.6	2

Figure S3: The Analytical Greenness report sheet for the HPLC-UV method.

Analytical Greenness report sheet

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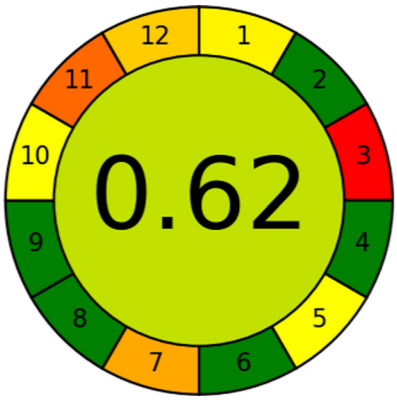

Criteria	Score	Weight
1. Direct analytical techniques should be applied to avoid sample treatment.	0.48	2
2. Minimal sample size and minimal number of samples are goals.	1.0	2
3. If possible, measurements should be performed in situ.	0.0	2
4. Integration of analytical processes and operations saves energy and reduces the use of reagents.	1.0	2
5. Automated and miniaturized methods should be selected.	0.5	2
6. Derivatization should be avoided.	1.0	2
7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.	0.33	2
8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	1.0	2
9. The use of energy should be minimized.	1.0	2
10. Reagents obtained from renewable sources should be preferred.	0.5	2
11. Toxic reagents should be eliminated or replaced.	0.2	2
12. Operator's safety should be increased.	0.4	2

Figure S4: The Analytical Greenness report sheet for the TLC-spectrodensitometric method.

Table S1: SM: Building the calibration curves and derivatized the regression equations for both medications

No.	The approach	The procedure for the regression equation
1)	Isoabsorpative point (Iso) [1,2]	The calibration curves were created by drawing the D0 absorbance for HYD at the isoabsorptive point, 256.2 nm (λ_{iso}), against the corresponding concentrations Using the solvent mixture as a blank, the second derivative, D2, was recorded for the CIP stored spectra ($\Delta\lambda$ = 8). Plotting the peak amplitude of the D2 spectra for CIP at 256.2 nm against the corresponding concentrations allowed for constructing a calibration curve
2)	Isoabsorpative point (Iso) method, with an absorbance ratio (AR); (Iso-AR) method [3]	Plotting the D0 absorbance for CIP at 330 nm against the appropriate concentrations for using the AR method. The calibration curve was created, and then the regression equation was calculated
3)	The extended ratio subtraction (EXRS) method [4,5] depends on the ratio subtraction (RS) method [6].	Plotting the D0 absorbance for CIP and HYD, respectively, against the corresponding concentrations at 278.6 and 243 nm
4)	The ratio difference (RD) method	The 5.0 µg/mL HYD spectrum divided the stored CIP spectra, and the 6.0 µg/mL CIP spectrum divided the HYD spectra. Then, plotting the difference between the amplitude ratio differences (ΔP279.8–241.7) and (ΔP241.7–221.5) for the CIP and HYD, respectively, against the corresponding concentrations allowed for the construction of calibration curves
5)	The mean centre concentrated ratio (MCR) method [4,7]	The MC for both medications were obtained by mean centering with respect to wavelength using the ratio spectra exported from the RD method to the Matlab platform. To create calibration curves, the maximum peak amplitude of MC for CIP and HYD, respectively, was plotted against the corresponding concentrations at 279.8 and 241.7 nm

Table S2: Theoretical aspects of UV-spectrophotometric manipulations for different approaches

No.	The approach	The manipulation theory
1)	Iso	Iso method, with a second derivative, for simultaneous determination of (*X*) and (*Y*) in their binary mixtures (µg/mL). The combination of drugs functions as a single unit. It provides the same absorbance value as a pure drug at the isoabsorpative point (λ_{iso}). Thus, the total concentration of both (*X*) and (*Y*) in the mixture could be calculated by measuring the absorbance value (A_{iso}) at the selected (λ_{iso}). In contrast, the concentration of (*X*) in the mixture could be calculated independently by using another spectrophotometric method. Therefore, by subtracting, the concentration of (*Y*) could be determined $A_{iso} = A_X + A_Y = a_{X_{iso}}C_X + a_{Y_{iso}}C_Y = a_{iso}(C_X + C_Y)$ at λ_{iso} (S1) Where $aX_{iso} = aY_{iso}$; $AX = aX\ C_X$; and Then $C_Y = (C_X + C_Y) - C_X$, for more details refer to reference [8]
2)	Iso-AR	Using the following equations, the AR establishes a linear relationship between the relative concentration of a binary mixture of (*X*) and (*Y*) and its absorbance ratio value: $C_X = \left(\frac{Q_1 - b_1}{a_1}\right)\left(\frac{A_{iso}}{a_{iso}}\right) \times 10^{-3}$ (S2) $C_Y = \left(\frac{Q_2 - b_2}{a_2}\right)\left(\frac{A_{iso}}{a_{iso}}\right) \times 10^{-3}$ (S3) C_X, C_Y = concentrations of drug *X* and *Y*, respectively; $Q_1 = A_1/A_{iso}$ for the first component (x); $Q_2 = A_2/A_{iso}$ for the second component (*Y*). At the isoabsorptive point ($A_{iso}/C_X + C_Y$), A_{iso} represents the absorbance and A_{iso} the absorptivity. The regression equation's slope, a_1, compares Q_1 to $C_X/C_X + C_Y$. The regression equation's slope, or a_2, is Q_2 versus $C_Y/C_X + C_Y$. The intercept values of these regression equations are b_1 and b_2. The mixture solution's absorbances at λ_1 and λ_2 are indicated by the letters A_1 and A_2. The concentration was changed from mg/mL to µg/mL by the (10^3), for more details refer to reference [8]
3)	EXRS	The proposed binary mixture of (*X*, CIP) and (*Y*, HYD), *X* concentration (µg/mL) can be determined by dividing the spectrum of the mixture (*X* + *Y*) by a known concentration of *Y* (µg/mL) as a divisor (*Y*'). A new curve representing $\left(\frac{X}{Y'}\right) + \left(\frac{Y}{Y'}\right)$ will result from this division, and show the plateau region where $\frac{Y}{Y'}$= constant may be measured. Then, a zero-order absorption spectrum D_0 of *X* (the original spectrum of *X*) could be obtained by subtracting this constant value and multiplying the obtained curve by *Y*' (the divisor), as the following equations show, for more details refer to reference [8]: $\frac{(X+Y)}{Y'} = \frac{X}{Y'} + \frac{Y}{Y'} = \frac{X}{Y'} + \text{constant.}$ (S4) $\frac{X}{Y'} + \text{constant} - \text{constant} = \frac{X}{Y'}.$ (S5) $\frac{X}{Y'} \times Y' = X$ as a zero−ordre absorption specta, D_0. (S6) Inversely, using *X*' as an advisor will extend the resulting equations to determine the second drug *Y*. Consequently, the regression equations that represent the linear relationship between the zero-order (D_0) absorbance at its λ_{max} and the corresponding varied concentrations of *X* and *Y*, respectively, could be used to evaluate the unknown concentrations of *X* and *Y* medications
4)	RD	This method relies on the interfering component's independence, i.e., the concentration of the component of interest is directly proportional to the amplitude difference between two points on the ratio spectra of a mixture. As a result, by dividing the mixture's spectrum by a known concentration of *Y* as a divisor (*Y*', µg/mL), the drug *X* (µg/mL) could be ascertained. The new curve that results from the division represents: $\frac{(X+Y)}{Y'} = \frac{X}{Y'} + \frac{Y}{Y'} = \frac{X}{Y'} + \text{constant.}$ (S7) To eliminate the constant $\frac{Y}{Y'}$ Furthermore, for any other instrumental error or interference from the sample matrix, choose two wavelengths (λ_1 and λ_2) on the obtained ratio spectrum and subtract the amplitudes at these two points.

(Continued)

Table S2: *Continued*

No.	The approach	The manipulation theory
		Assuming that the amplitudes at the two chosen wavelengths are $P1$ and $P2$ at λ_1 and λ_2, respectively, the interfering substance Y exhibits no interference when the two amplitudes are subtracted $P1 - P2 = \left[\left(\frac{Y}{Y'}\right)_1 + \text{constant}\right] - \left[\left(\frac{Y}{Y'}\right)_2 + \text{constant}\right] = \left(\frac{Y}{Y'}\right)_1 - \left(\frac{Y}{Y'}\right)_2.$ (S8) The regression equation, which shows the linear relationship between the variations in ratio spectra amplitudes at the two chosen wavelengths and the corresponding drug (X) concentration (μg/mL), is used to determine the concentration of X. Likewise, by applying the same process to a known concentration of X (μg/mL) as a divisor X', Y could be ascertained, for more details refer to reference [8]
5)	MCR	The ratio spectra are obtained in this manner, and the next step is to mean centre the ratio spectra to eliminate the constant. It can be written if there is no interaction between the compounds. Beer's law is followed for every compound in the combination of the drugs (X) and (Y). $A_{\mathrm{m}=ax C_X + C_Y}$ (S9) Where A_{m} is the mixture's absorbance vector, a_X and a_Y are X and Y's molar absorptivity vectors, and C_X and C_Y are X and Y's respective concentrations. Assume that A_{m} is split by αY, representing the spectrum of a typical Y solution in a binary mixture. The first ratio spectrum is then obtained as follows in that scenario: $B = \frac{A_{\mathrm{m}}}{a_Y} = \frac{a_X C_X}{a_Y} + C_Y$ (S10) Given that a constant's (C_Y) mean-centering is zero, B is said to be mean-centered (MC) $\mathrm{MC}(B) = \mathrm{MC}\left(\frac{A_{\mathrm{m}}}{a_Y}\right) = \mathrm{MC}\left(\frac{a_X C_X}{a_Y}\right)$ (S11) Without affecting (interfering) the other compound (Y), it can be seen that there is a linear relationship between the concentration of (X) in the solution and the amount of MC(B). Similarly, (Y) could be found by dividing A_{m} by a_X, which would be the spectrum of a typical solution of (X), and then continue as previously, for more details refer to reference [8]

Table S3: Chromatographic conditions and trails on HPLC-UV and TLC-spectrodensitometric methods

No	The studied point	The utilized approach	
		RP-HPLC-UV	**TLC-spectrodensitometric**
1)	The selected wavelength, nm	– The CIP and HYD together constituted a ratio of (2.3:10) in their commercial dosage forms. Both medications responded equally when the detection was made at 256 nm (Figure 2b, in the manuscript. For more details refer to reference [9]	– Different scanning wavelengths, 243 and 256 nm, have been tried. – Nevertheless, the separated peaks were more symmetrical, noise-free, and sharp at 243 nm. For more details refer to reference [9]
2)	The separated stationary phase	– According to the initial tests, both medications had a higher resolution on a Zorbax SB-C_{18} column – However, the two medications could not be separated using a Zorbax SB-C_8 column	– Only one stationary phase was tested: a TLC aluminium sheet (20 × 20 cm) that was precoated with silica gel 60 F254
3)	Mobile phase	– Several bidistilled water-to-acetonitrile ratios were examined. – The separation of CIP was marginally impacted by increasing the acetonitrile ratio – However, it shortened the retention time of HYD, which impacted its elution and resulted in the overlap of HYD's peak with CIP's – There was some broadening of the CIP peak with an increase in the water ratio – The HYD peak tails when methanol is used in place of acetonitrile	– The separation of CIP was somewhat impacted by increasing the ethyl acetate ratio. It did, however, also have an impact on HYD's migration to the solvent front. Hexane was added to stop HYD from migrating to the solvent front, but it also suppressed the migration of CIP – It took triethylamine to lessen both peaks' tailing – Nevertheless, HYD moved to the solvent front when the ratio (or any other polar solvent, like methanol, ethanol, etc.) was increased – Ultimately, it was discovered that employing the developing system with ethyl acetate, hexane, and triethylamine (50: 25: 25 v/v/v) produced the best separation of CIP and HYDHYD
4)	pH-controlled mobile phase	– The addition of the pH-controlled solution preserved the CIP's peak symmetry – Acetonitrile: bidistilled water: pH-controlled solution (pH 3) in the ratio (55: 40: 5 v/v/v) was used as the mobile phase	- Triethylamine was added to adjust the pH to alkaline
5)	Retention time, minutes	– Each sample was run for less than 6 minutes – The selected retention times for CIP and HYD were 1.97 and 5.58, respectively, (Figure 2b, in the manuscript. For more details refer to reference [9]	– As illustrated in (Figure 2c, in the manuscript. For more details refer to reference [9]., CIP and HYD were separated at Rf 0.06 ± 0.02 and 0.66 ± 0.01, respectively, due to the significant polarity difference between the two medications.
6)	Flow rate	– Various flow rates (0.5–1.2 mL/min) and wavelengths (243 and 256 nm) were tested – At 256 nm, good resolution and linearity were achieved with 0.6 mL/min	- The developing solvent system was ascended by capillary action. - TLC plates were scanned for both drugs at 243 nm. The detection was performed with the Camage TLC scanner 3

Table S4: KPIs-based standards were used in benchmarking for the degree whiteness of the developed analytical approaches using the RGB 12 Algorithm

Algorithm	KPIs-based standards
Red	*R1 (Scope of application):* number of simultaneously determined analytes, linearity range, resistance to the presence of potential interferences, Applying of Analytical Quality by Design
	***R**2 (LOD & LOQ):* limits of detection and quantification (LOD and LOQ)
	***R**3 (Precision):* expressed in repeatability and reproducibility of the results
	***R**4 (Accuracy):* expresses as minimal relative error of determinations
Green	*G1 (Toxicity of reagents):* number of GHS Hazardous pictogram
	***G**2 (reagents and waste):* expressed as reagent consumption and waste production
	***G**3 (Energy):* lowest possible consumption of electricity
	***G**4 (Direct impact):* safety of operator, use of animals or GMOs
Blue	*B1 (Cost Efficiency):* related to personnel training level
	***B**2 (Time-efficiency):* total time of analysis
	***B**3 (Requirements):* includes sample consumption, advanced equipment, personnel qualifications, and laboratory infrastructure.
	***B**4 (Operational simplicity):* includes portability, integrated automation and miniaturization

Table S5: KPIs-based standards were used in benchmarking the greenness of the developed analytical methods

Utilized tool	KPIs-based standards		
AES	(1) sample preparation, (2) reagent and compound used, (3) sample collection, (4) preservation, transport, and storage, (5) instrumentation, and finally (6) method type		
AGREE	(1) sample treatment, (2) sample amount, (3) device positioning, (4) sample preparation, (5) automated and miniaturized methods, (6) derivatization, (7) generation of a volume of waste, (8) analysis throughput, (9) use of energy, (10) renewable source of reagent, (11) toxicity of reagents, and finally (12) safety of the operator		
GAPI	**Sample preparation and analysis**		
	I. Samples preparation	**II. Reagents and solvents**	**III. Instrumentation**
	(1) their collection, (2) preservation, (3) transport, (4) storage, (5) type of method, (6) scale of extraction, (7) solvents/reagents used, and finally (8) additional treatment	(1) their amount, (2) health hazards, and finally, (3) safety hazards	(1) their consumed energy, (2) occupational hazard, (3) waste, and finally, (4) waste treatment

Table S6: KPIs-based standards were used in benchmarking the blueness of the developed analytical approaches

Utilized tool	KPIs-based standards
BAGI	(1) Type of analyte
	(2) Single or multiple-element analysis
	(3) Analytical approach
	(4) Simultaneous sample preparation
	(5) Sample preparation
	(6) Samples pre-hour
	(7) Reagent and materials
	(8) Preconcentration,
	(9) Automation degree
	(10) Amount of sample.

Table S7: Computational spectrum manipulation techniques to resolve the overlapping zero-order absorption spectra (D^0) of HYD and CIP

No	The manipulated approach	Manipulation steps
1)	Iso	– The total concentration (μg/mL) of CIP plus HYD has been determined using the regression equation that illustrates the linear relationship between the D_0 absorbance of HYD at 256.2 nm and the Iso point (λ_{iso} = 256.2 nm), as shown in Figure S7 – Moreover, the linear relationship between the D_2 absorbance of CIP at 256.8 nm and the regression equation could be used to determine the concentration of CIP in the mixture without any interference. Consequently, HYD concentration was determined by subtracting
2)	AR	– Utilizing the obtained linear relationship between the AR value of the binary mixture of CIP (X) and HYD (Y) and the relative concentration (μg/mL) in that mixture, the AR method was utilized to ascertain the concentration of CIP (C_x) and HYD (C_y) in a binary mixture (μg/mL) – The choice of ideal wavelengths and the presence of an isoabsorpative point are the two primary requirements for using the AR method – To achieve a high degree of separation, the technique should be used with wavelengths (λ_1, λ_2, λ_{iso}) that allow for accurate component determination in the binary mixture – For identifying synthetic mixture concentrations of CIP and HYD, selecting two wavelengths (330 and 243 nm) adjacent to the isoabsorpative point (256.2 nm) produced the best recovery percentages. – Plotting the absorbance of the previously synthetic mixture at λ_{iso} (256.2 nm) against the total concentrations of (C_X) and (C_Y) was done. – The slope of the regression line (A_{iso} is 0.030) was calculated using the regression equation. At 330 nm, 243 nm, and 256.2 nm, the absorbance of each solution was measured. – The relative absorbance (Q_1 is A_1/A_{iso}) was then plotted against the relative concentration ($C_X/(C_X + C_Y)$. The parameters of the regression equation (b_1/a_1 is 0.071) were calculated. – Plotting the relative absorbance ($Q_2 = A_2/A_{iso}$) against the relative concentration ($C_Y/(C_X + C_Y)$ allowed for the computation of the regression equation parameters (b_2/a_2 is 0.649).
3)	EXRS	– The EXRS method initially started as an extension of the well-known spectrum manipulation of the RS method – The mixture's spectrum has been divided using a specific concentration (μg/mL) of one component as a divisor (a constant value will be the result). Therefore, the other has to be determined quantitatively – The absorbance of the constant value is then calculated along the plateau region parallel to the baseline at ≥ ± 2 nm (corresponding to the divisor's maximum peak in its zero order), subtracted from the ratio spectra, and finally multiplied by the divisor – To minimize noise error, each component of interest is recovered in its zero-order spectra and measured at its λ_{max} in the RS and EXRS methods. By dividing the binary mixture's spectrum by a known concentration of CIP as a divisor (6.0 μg/mL of CIP), the concentration of HYD could be assessed – The division's constant was measured at the plateau (330–350 nm). We can recover the zero-order absorption spectrum of HYD present in the mixture, Figure S8, by subtracting this constant value and multiplying the obtained curve by the spectrum of 6.0 μg/mL of CIP (the divisor). The measurement was made at its λ_{max} (243 nm). – Next, to calculate the concentration of CIP in the mixture, the constant value at (225–255 nm) was obtained by dividing the obtained D_0 spectrum of a known concentration of HYD as a divisor (5 μg/mL). – The previously obtained constant can be obtained by dividing the binary mixture's spectrum by the divisor, or 5.0 μg/mL of HYD.

(Continued)

Table S7: *Continued*

No	The manipulated approach	Manipulation steps
		– After subtracting this constant, we can obtain the zero-order absorption spectrum of CIP present in the mixture (Figure 9 and measure it at its λ_{max} (278.6 nm) by multiplying the obtained curve by the spectrum of 5.0 µg/mL of HYD. – Furthermore, the linear relationship between each compound's zero order (D_0) absorbance at 278.6 and 243 nm and the corresponding concentrations of CIP and HYD was determined using regression equations to determine the concentration of CIP and HYD, respectively – Various concentrations of HYD (3.0, 5.0 and 7.0 µg/mL) and CIP (3.0, 6.0 and 9.0 µg/mL) were tested as a divisor; however, the concentrations of HYD (5.0 µg/mL) and CIP (6.0 µg/mL) produced the smoothest RS, the least amount of noise, and the highest sensitivity
4)	RD	– The RD method uses the ratio spectrum to measure the difference between two amplitude values at two wavelengths in a single step, reducing the need for derivative steps and improving signal-to-noise ratio – To guarantee a low noise-to-signal ratio at these wavelengths, the linearity of the amplitude values at each selected wavelength against the corresponding concentration should be examined – To eliminate noise, the two values at two chosen wavelengths are subtracted when using the RD method to measure the difference between the amplitude values – The contribution of the CIP and HYD at the two chosen wavelengths (279.8 and 241.7 nm, in this case) was the only requirement for determining the CIP concentration using this method – The HYD's ratio spectrum displayed the same amplitudes (constant). However, the CIP showed a significant difference in these two amplitude values with concentration at these two chosen wavelengths. Likewise, two additional wavelengths (241.7 and 221.5 nm) were chosen to estimate the HYD – The spectra of HYD (5.0 µg/mL) and CIP (6.0 µg/mL) divided the spectra of CIP solutions (2.0–14.0 µg/mL) and HYD solutions (1.0–14.0 µg/mL), respectively, as shown in Figure S10. – The linear relationship between the differences of these ratio spectra amplitudes at the two chosen wavelengths and the corresponding concentration of drug CIP was represented by the regression equation, which was used to calculate the concentration of CIP. Likewise, HYD might be ascertained in the same way. The concentrations of the divisors were chosen as per the EXRS method. – When used to predict CIP and HYD concentrations in bulk powder and mixture, the chosen wavelengths yielded the best average recovery percentage
5)	MCR	– By doing away with derivative steps, this approach improves the signal-to-noise ratio. – The ratio spectra for both drugs from the RD method were mean-centred in the 200–330 nm range, as shown in Figure S11. The spectra from 330–400 nm were excluded because they impacted the MC curves' linearity – The regression equation, which shows the linear relationship between (MC) at 279.8 nm for CIP and 241.7 nm for HYD, was used to determine the concentration of both medications (µg/mL)

Table S8: Statistical analysis of parameters required for system suitability of HPLC-UV and TLC-spectrodensitometric methods

Parameter	HPLC-UV method		TLC-spectrodensitometric method		Reference value [10]
	CIP	HYD	CIP	HYD	
t_R (HPLC-UV)/ R_f (TLC-spectrodesitometry)	1.97	5.58	0.06 ± 0.01	0.66 ± 0.01	$t_R > 1$ (HPLC-UV)
N (Column efficiency)	7096	13960			$N > 2000$ Increases the efficiency of the separation.
HETP (Height equivalent to theoretical plates)	0.002	0.001			The smaller the value, the higher the column efficiency.
T (tailing factor)	1.03	0.89	1.13	1.01	$T < 2$. $T = 1$ for symmetric peak
Rs (Experimental Resolution)	8.30		10.48		Rs > 2.

Table S9: Assay parameters and method validation were obtained by applying UV-spectrophotometric manipulated methods

Parameters	Iso method		AR method	EXRS method		RDS method		MCR method	
	CIP D^2 (256.8 nm)	HYD D_0 (256.2 nm)	CIP D_0 (330 nm)	CIP D_0 (278.6 nm)	HYD D_0 (243 nm)	CIP $P1 - P2$ (279.8 –241.7)	HYD $P1 - P2$ (241.7–221.5)	CIP MC (279.8 nm)	HYD MC (241.7 nm)
Calibration range (n = 5)	2.0–14.0	1.0–14.0	2.0–14.0	2.0–14.0	2.0–14.0	2.0–14.0	2.0–14.0	2.0–14.0	1.0–14.0
LOD, µg/mL	0.19	0.16	0.13	0.14	0.08	0.18	0.12	0.13	0.05
LOQ, µg/mL	0.58	0.50	0.40	0.41	0.26	0.56	0.37	0.40	0.16
Slope	0.0327	0.0301	0.0305	0.1044	0.0512	6.3589	0.2654	3.8564	0.3208
Intercept	−0.0039	0.0010	0.0032	0.0042	−0.0009	0.6035	−0.0041	0.2318	−0.0087
Mean % (n = 3)	100.18	99.93	99.99	100.20	100.16	100.04	99.89	100.07	99.93
RSD (n = 3)	0.83	0.59	0.69	0.87	1.01	0.82	0.60	0.75	0.46
Accuracy (Mean recovery % ± RSD, n = 3).	99.96 ± 1.65	99.58 ± 0.58	100.21 ± 1.34	100.57 ± 0.87	99.92 ± 0.47	100.22 ± 1.17	99.52 ± 0.32	100.03 ± 1.13	100.16 ± 0.48
Intra-day precision (Mean recovery % ± RSD, n = 3)	0.60	0.43	0.97	0.61	0.16	0.42	0.27	0.47	0.57
Inter-day precision (Mean recovery % ± RSD, n = 3).	0.48	0.84	1.02	0.83	0.44	0.54	0.49	0.66	0.84
Robustness (Mean recovery % ± RSD, n = 3)	0.91	1.01	0.93	0.76	0.64	0.91	0.55	0.73	0.84
Correlation coefficient (r)	0.9999	0.9999	0.9999	1.00	1.00	0.9999	0.9999	0.9999	1.00

D^2 is the second derivative amplitude; MC is the mean centring amplitude; $P1$–$P2$ is the difference in amplitude; and D_0 is the zero-order absorbance.

Table S10: Assay parameters and method validation were obtained by applying the RP-HPLC-UV and TLC-spectrodensitometric methods

Parameters	RP-HPLC-UV method		TLC-spectrodensitometric method	
	CIP	HYD	CIP	HYD
Calibration range[a] (*n* = 5)	1.0–8.0	1.0–8.0	0.2–1.6	0.6–2.0
LOD, µg/Ml	0.12	0.08	0.03	0.05
LOQ, µg/mL	0.37	0.24	0.09	0.14
Slope	0.8741	0.9405	2.969	3.593
Intercept	0.1235	0.0304	2.208	0.2453
Mean %, (*n* = 3)	99.89	100.28	99.74	99.94
RSD (*n* = 3)	0.61	1.27	1.27	1.54
Accuracy (Mean recovery % ± RSD, *n* = 3).	100.32 ± 0.78	99.89 ± 0.34	100.50 ± 1.00	100.21 ± 0.59
Intra-day precision (Mean recovery % ± RSD, *n* = 3)	100.37 ± 0.19	99.86 ± 0.46	100.29 ± 0.65	99.78 ± 0.76
Inter-day precision (Mean recovery % ± RSD, *n* = 3).	99.89 ± 0.40	99.83 ± 0.45	100.20 ± 1.01	99.29 ± 1.15
Robustness (Mean recovery % ± RSD, *n* = 3).	100.32 ± 0.42	99.95 ± 0.61	100.94 ± 1.28	100.43 ± 1.73
Correlation coefficient (*r*).	0.9999	0.9999	0.9998	0.9997

[a] RP-HPLC-UV methods: in (µg/mL); TLC-spectrodensitometric methods: in (µg/band).

Table S11: Determination of CIP and HYD in laboratory-prepared mixtures by the UV-spectrophotometric manipulated methods

Ratios	Iso method		AR method		EXRS method		RDS method		MCR method	
	CIP	HYD	CIP	HYD	CIP	HYD	CIP	HYD	CIP	HYD
	Recovery % [a]									
5:5	98.40	101.60	98.65	98.88	99.65	99.96	99.01	99.56	99.00	101.40
2:4	102.50	101.75	102.31	101.05	102.44	101.51	99.88	99.86	100.00	101.25
2:6	101.00	102.00	99.30	98.47	101.49	99.90	99.20	101.43	99.50	101.17
2:1	99.00	101.70	100.35	99.64	101.49	100.37	101.41	100.72	101.50	101.19
8:4	102.00	100.75	100.39	100.42	100.99	101.51	100.71	98.62	100.63	101.75
4:6	101.50	99.50	100.16	99.46	101.32	100.88	100.20	99.48	100.25	102.00
9:3	100.89	99.33	100.19	102.18	100.75	101.50	100.60	99.59	100.56	101.67
6:4	101.33	102.75	102.25	101.77	101.74	102.00	102.25	98.82	102.00	101.25
Mean ± SD	100.83	101.17	100.45	100.23	101.23	100.95	100.41	99.76	100.43	101.55
(*n* = 3)	± 1.42	± 1.21	± 1.28	± 1.35	± 0.82	± 0.80	± 1.09	± 0.93	± 0.99	± 0.32

Table S12: Determination of CIP and HYD in laboratory-prepared mixtures by the HPLC-UV and TLC-spectrodensitometric methods

Mixture No.	Ratio	HPLC-UV method						TLC-spectrodensitometric method					
		CIP			HYD			CIP			HYD		
		Taken (µg/mL)	Found[a] (µg/mL)	Recovery%	Taken (µg/mL)	Found[a] (µg/mL)	Recovery%	Taken (µg/mL)	Found[a] (µg/mL)	Recovery%	Taken (µg/mL)	Found[a] (µg/mL)	Recovery%
1	1:1	2	2.01	100.54	2	1.99	99.54	0.80	0.80	100.32	0.80	0.80	99.77
2	1:2	2	1.99	99.69	4	4.03	100.69	0.40	0.40	100.98	0.80	0.80	99.65
3	1:3	1	1.01	100.80	3	2.99	99.66	0.20	0.20	101.43	1.60	1.61	100.63
4	1:5	1	1.01	101.18	5	5.00	100.01	0.20	0.20	100.32	1.00	1.01	100.93
5	2:1	4	4.04	100.93	2	2.01	100.59	1.2	1.20	99.76	0.60	0.61	101.32
6	2:3	2	1.99	99.64	3	2.99	99.90	0.60	0.60	99.65	0.90	0.90	100.02
7	3:2	3	2.99	99.79	1	1.00	99.72	0.90	0.91	100.82	0.60	0.60	99.84
Mean ± SD			100.38 ± 0.60			100.11 ± 0.50			100.47 ± 0.65			100.31 ± 0.61	

[a]n = 3.

Table S13: Application of standard addition technique to the analysis of CIP and HYD in the commercial ear drops by UV-spectrophotometric manipulated methods benchmarked to the manufacturer method [11]

Methods	CIP		HYD	
	Found% ± SD (*n* = 6)	**Pure added (2.0, 4.0, 6.0 µg) mean recovery% ± SD (*n* = 3)**	**Found% ± SD (*n* = 6)**	**Pure added (1.0, 2.0, 3.0 µg) mean recovery% ± SD (*n* = 3)**
Iso	101.23 ± 0.58	100.60 ± 0.18	98.07 ± 0.46	100.34 ± 0.12
AR	101.24 ± 0.81	100.56 ± 0.62	98.03 ± 0.74	100.43 ± 0.55
EXRS	101.28 ± 0.62	100.43 ± 0.42	98.35 ± 0.66	99.87 ± 0.74
RDS	101.33 ± 0.94	100.52 ± 0.19	98.75 ± 0.73	100.19 ± 0.27
MCR	100.90 ± 0.93	100.16 ± 0.23	98.11 ± 0.78	100.49 ± 0.36
Manufacturer HPLC (11)*	101.66 ± 0.81		98.01 ± 0.64	

*Manufacturer method [11] is HPLC-UV (the conditions: RP $_{C18}$ column, mobile phase (buffered bidistilled H_2O: MeOH, 55:45%, v/v), the flow rate = 1.5 mL/min, and the detection at 240 nm).

Table S14: Application of standard addition technique to the analysis of CIP and HYD in the commercial ear drops by the RP-HPLC-UV and TLC-spectrodensitometric methods benchmarked to the manufacturer method [11]

Commercial ear drops	RP-HPLC-UV method					TLC-spectrodensitometric method					Manufacturer method [11][c]
	Claimed (µg/mL)	Found (µg/mL)	Recovery%[a]	Pure added (µg/mL)	Recovery%[b]	Claimed (µg/band)	Found (µg/band)	Recovery%[a]	Pure added (µg/band)	Recovery%[b]	Recovery%[a]
CIP	1.15	1.16	101.20 ± 0.48	2.0	2.01	0.23	0.23	101.48 ± 0.51	0.5	100.96	101.66 ± 0.81
				3.0	3.04				0.75	100.43	
				4.0	4.01				0.85	100.13	
				5.0	4.98				1.0	99.99	
		Mean ± SD		100.40 ± 0.73					100.15 ± 0.41		
HYD	5.0	4.91	98.14 ± 0.36	1.0	1.00	1.0	0.982	98.25 ± 0.34	0.2	0.20	98.01 ± 0.64
				1.5	1.50				0.4	0.40	
				2.0	2.00				0.6	0.60	
				2.5	2.53				0.8	0.80	
		Mean ± SD		100.27 ± 0.55					100.25 ± 0.41		

[a]$n = 6$. [b]$n = 3$. [c]Manufacturer method [11] is RP-HPLC-UV (the conditions: RP $_{C18}$ column, mobile phase (buffered bidistilled H_2O: MeOH, 55:45%, v/v), the flow rate = 1.5 mL/min, and the detection at 240 nm).

Table S15: Statistical comparison between the results obtained by the UV-spectrophotometric manipulated methods and official BP methods (12) for the determination of CIP and HYD in pure powder form

Items	CIP determination methods						HYD determination methods				
	Iso	AR	EXRS	MCR	RDS	Official method	Iso	EXRS	RDS	MCR	Official method
Mean recovery% (n = 5)	99.91	99.86	100.20	100.07	100.04	100.82	99.93	100.16	99.89	99.93	99.46
RSD	1.14	0.30	0.76	0.58	0.67	0.19	0.21	1.17	0.36	0.17	0.26
Standard error of the mean, SEM	0.29	0.33	0.25	0.38	0.38	0.33	0.19	0.27	0.21	0.32	0.26
No. of experiments	5	5	5	5	5	5	5	5	5	5	5
Student's t-test (2.201)**	1.539	2.192	1.471	1.991	1.922		1.479	1.417	1.317	1.768	
F value (6.094)**	3.583	1.566	3.909	2.943	3.497		1.331 (4.120)**	3.949	1.408	1.212 (4.120)**	

**Figures between parentheses represent the corresponding tabulated values of the t-test and F-test at p = 0.05.

Table S16: SM. Statistical comparison between the results obtained by the RP-HPLC-UV and TLC-spectrodensitometric methods and official BP methods [12] for the determination of CIP and HYD in pure powder form

Parameters	RP-HPLC-UV methods		TLC-spectrodensitometric methods		Official method	
	CIP	HYD	CIP	HYD	CIP	HYD
Mean recovery% (n = 5)	99.89	100.28	99.74	99.94	99.86	100.52
RSD	0.61	1.27	1.27	1.54	0.85	0.64
No. of experiments	5	5	5	5	5	5
Student's t-test (1.81)*	1.09	0.87	1.32	1.01		
F test (4.53)*	2.03	2.44	1.15	3.17		

*Figures between parentheses represent the corresponding tabulated values of the t-test and F-test at p = 0.05.

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