Simultaneous Determination of Losartan and Hydrochlorothiazide in Combined Dosage Forms by First-Derivative Spectroscopy and High-Performance Thin-Layer Chromatography

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Losartan (LST) is the first orally active nonpeptide angiotensin-II receptor antagonist with an improved safety and tolerability profile. It is prescribed alone or in combination with hydrochlorothiazide (HCTZ) for the treatment of moderate-to-severe hypertension. This paper describes the development of 2 methods that use different techniques, first-derivative spectroscopy and high-performance thin-layer chromatography (HPTLC), to determine LST and HCTZ in the presence of each other. LST and HCTZ in combined preparations were quantitated by using the first-derivative responses at 271.6 nm for LST and 335.0 nm for HCTZ in spectra of their solutions in water. The linearity ranges are 30–70 μg/mL for LST and 7.5–17.5 μg/mL for HCTZ with correlation coefficients of 0.9998 and 0.9997, respectively. In the HPTLC method, a mobile phase of chloroform–methanol–acetone–formic acid (7.5 + 1.5 + 0.5 + 0.03, v/v) and a prewashed Silica Gel G60 F_{254} TLC plate as the stationary phase were used to resolve LST and HCTZ in a mixture. Two well-separated and sharp peaks for LST and HCTZ were obtained at Rf values of 0.61 ± 0.02 and 0.41 ± 0.02, respectively. LST and HCTZ were quantitated at 254.0 nm. The linearity ranges obtained for the HPTLC method are 400–1200 and 100–300 ng/spot with corresponding correlation coefficients of 0.9944 and 0.9979, for LST and HCTZ, respectively. Both methods were validated, and the results were compared statistically. They were found to be accurate, specific, and reproducible. The methods were successfully applied to the estimation of LST and HCTZ in combined tablet formulations.

Losartan (LST) is the potassium salt of 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)-methyl-imidazole. It is used for the treatment of hypertension and is the prototype of the new generation of potent, orally active, nonpeptide angiotensin-II antagonists (1–4). Hydrochlorothiazide (HCTZ) is a diuretic that is often combined with other antihypertensive agents (5).

The combination of LST and HCTZ has been recently introduced in the market for the treatment of moderate-to-severe hypertension.

The analytical techniques reported for the determination of LST include liquid chromatography (LC; 6–9), high-performance thin-layer chromatography (HPTLC; 10), capillary electrophoresis (6), supercritical fluid chromatography (6), etc. LC is the most widely used technique, but it is time consuming and comparatively expensive.

Several analytical methods that use spectrophotometry (11–14), LC (14–18), and HPTLC (19) have been reported in the literature for the determination of HCTZ in pharmaceutical preparations, either separately or in combination with other drugs.

This paper presents a first-derivative spectroscopic method and an HPTLC method for the simultaneous determination of LST and HCTZ in mixtures without prior separation. Also, the utility of the proposed methods for the determination of both drugs in pharmaceutical formulations is demonstrated.

Experimental—First-Derivative Spectroscopy

Apparatus

A double-beam Shimadzu 160A UV-Visible spectrophotometer having 2 matched quartz cells with a 1 cm lightpath was used for spectroscopic analysis.

Reagents and Materials

Analytically pure samples of LST and HCTZ were a gift from Cadila Pharmaceuticals Ltd. (Ahmedabad, India). Analytical reagent-grade methanol (Sisco Research Laboratory Chem.) and distilled water were used for the preparation of solutions. Tablet formulation X was procured from the local market, and formulation Y was a gift from Intas Pharmaceuticals Ltd. (Ahmedabad, India).

Preparation of Standard Solutions

(**a**) *LST standard solution*.—A 1 mg/mL stock solution of LST was prepared in methanol. An appropriate volume of

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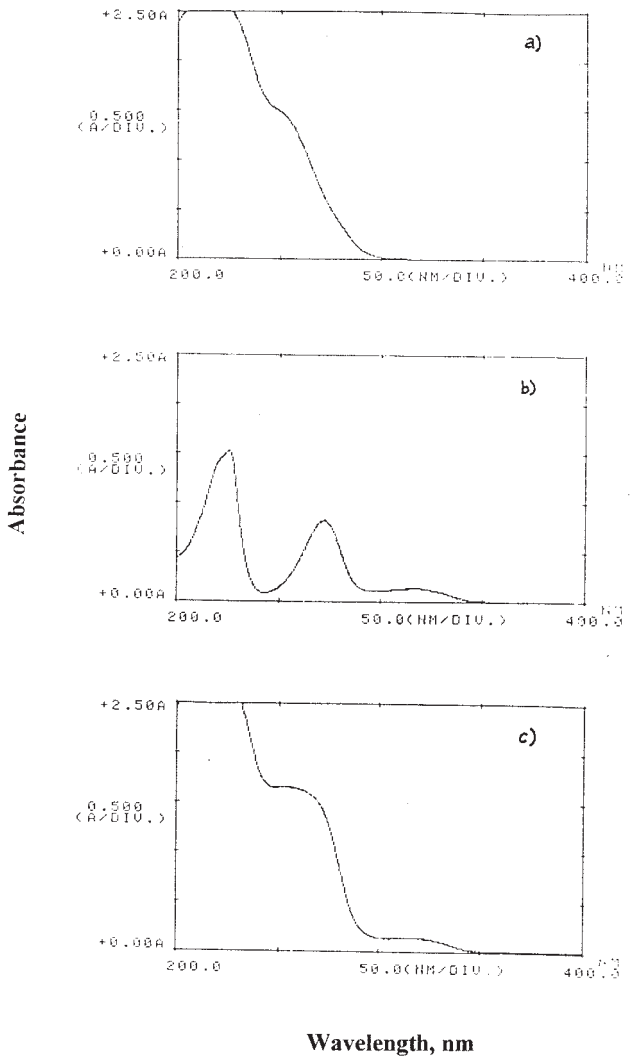

Figure 1. Zero-order spectra of (a) LST standard solution (50 μg/mL), (b) HCTZ standard solution (12.5 μg/mL), and (c) a mixture of standard solutions of LST (50 μg/mL) and HCTZ (12.5 μg/mL) in methanol.

stock solution was further diluted with water to obtain a standard solution of LST having a final concentration of 200 μg/mL.

(**b**) *HCTZ standard solution*.—A 1 mg/mL stock solution of HCTZ was prepared in methanol. An appropriate volume was diluted suitably with water to obtain a standard solution of HCTZ having a final concentration of 50 μg/mL.

Selection of Wavelengths for Estimation of LST and HCTZ

Standard solutions of LST and HCTZ were diluted appropriately with water to obtain an LST concentration of 50 μg/mL and an HCTZ concentration of 12.5 μg/mL. Similarly, appropriate volumes of LST and HCTZ standard solutions were mixed and suitably diluted with water to achieve a mixture containing LST at 50 μg/mL and HCTZ at 12.5 μg/mL. Spectra of these diluted solutions were scanned

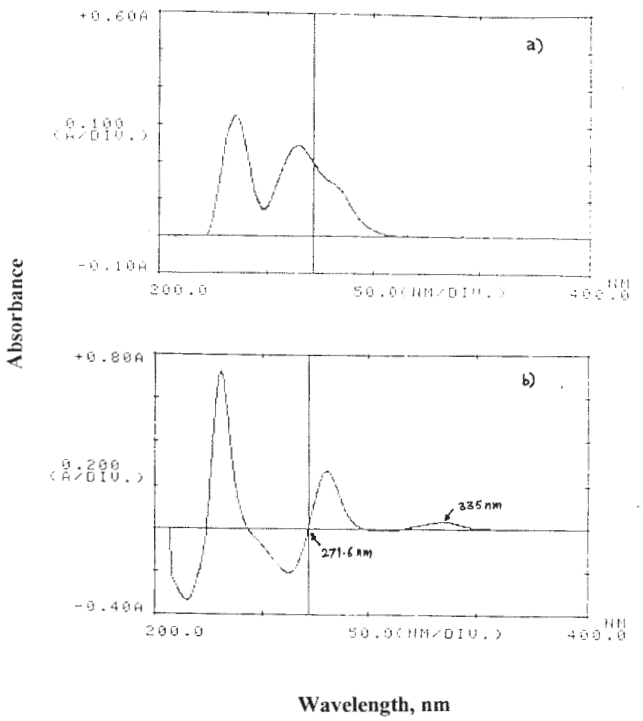

Figure 2. First-derivative spectra of (a) LST standard solution (50 μg/mL) and (b) HCTZ standard solution (12.5 μg/mL).

in the Spectrum basic mode between 200 and 400 nm versus water as a blank. These zero-order spectra of LST, HCTZ, and their mixtures were treated to obtain corresponding first-order derivative spectra.

Derivative Conditions

The first-order derivative spectra were scanned by using the Spectrum mode (slit width = 1.4 × 16 mm) with fast (μ 2400 nm/min) scan speed. The derivative spectra were recorded by using digital differentiation (convolution method) with 17 to 25 data points with a derivative wavelength difference ($\Delta\lambda$) of 12.0 nm in the range of 200–400 nm. No smoothing of the spectra was found to be necessary.

The first-derivative spectra were overlapped by using memory channels. The zero-crossing points (ZCPs) of HCTZ at which the LST showed some derivative response were recorded. The wavelength 271.6 nm was selected for the quantitation of LST (where the derivative response for HCTZ is zero). Similarly, 335.0 nm was selected for the quantitation of HCTZ (where the derivative response for LST is zero).

Characteristic wavelengths (ZCPs) for LST and HCTZ were confirmed by varying the concentration of one component while the concentration of the other component was kept constant, and vice versa.

Calibration Curves for LST and HCTZ

The standard solutions of LST (200 μg/mL) and HCTZ (50 μg/mL) were used to prepare 5 different sets of dilution, Series A–E, as follows:

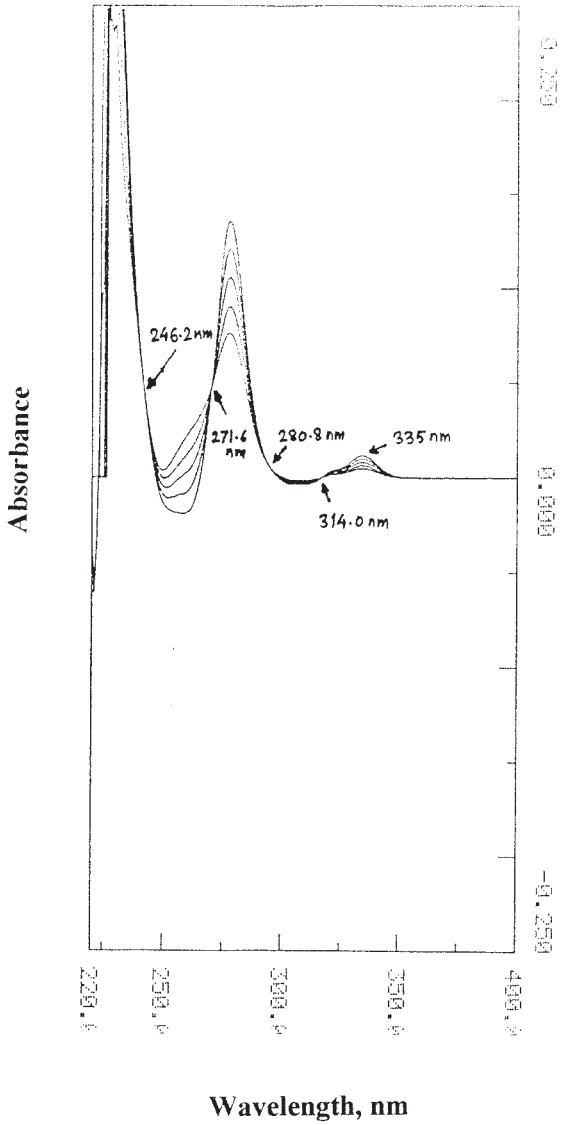

Figure 3. First-derivative spectra of mixtures containing HCTZ (7.5, 10, 12.5, 15, and 17.5 μg/mL) with a fixed concentration of LST (50 μg/mL; recorded by using a Jasco 7850 UV-Vis spectrophotometer).

Series A.—This series consisted of LST solutions of various concentrations (30–70 μg/mL) prepared by pipetting appropriate volumes (1.5, 2.0, 2.5, 3.0, and 3.5 mL) of LST standard solution (200 μg/mL) into 10 mL volumetric flasks and diluting to volume with water.

Series B.—This series included solutions with different concentrations of HCTZ (7.5–17.5 μg/mL) prepared by diluting appropriate volumes of HCTZ standard solution (50 μg/mL) as described for Series A.

Series C.—This series consisted of mixtures of LST and HCTZ solutions, having a fixed concentration of HCTZ (12.5 μg/mL) and various concentrations of LST (30–70 μg/mL). The solutions were prepared by mixing 1.5, 2.0, 2.5, 3.0, and 3.5 mL LST standard solution (200 μg/mL) with 2.5 mL HCTZ standard solution (50 μg/mL) in a series of 10 mL volumetric flasks and diluting to volume with water.

Series D.—In this series, mixtures of LST and HCTZ solutions having a fixed concentration of LST (50 μg/mL) and various concentrations of HCTZ (7.5–17.5 μg/mL) were prepared by mixing 2.5 mL LST standard solution (200 μg/mL) with 1.5, 2.0, 2.5, 3.0, and 3.5 mL HCTZ standard solution (50 μg/mL) in a series of 10 mL volumetric flasks and diluting to volume with water.

Series E.—In this series, mixtures of LST and HCTZ solutions having various concentrations of LST (30–70 μg/mL) and HCTZ (7.5–17.5 μg/mL) were prepared by mixing appropriate volumes of the corresponding standard solutions in a series of 10 mL volumetric flasks and diluting to volume with water.

The derivative responses of the solutions of Series A, C, and E were measured at 271.6 nm to prepare calibration curves for LST; the derivative responses of the solutions of Series B, D, and E were measured at 335.0 nm to prepare calibration curves for HCTZ.

Method Validation

The method was validated for precision, linearity, accuracy, and specificity.

Precision.—The intraday and interday precision of the proposed first-derivative spectroscopy method were determined by estimating the corresponding response at 3 times on the same

Table 1. Calibration curve data for the determination of LST and HCTZ in the presence of each other (Series E) by the proposed first-derivative spectroscopy method

Series E composition, μg/mL					
LST	HCTZ	D_1[a] for LST at 271.6 nm	CV, %[b]	D_1[a] for HCTZ at 335.0 nm	CV, %[b]
30	7.5	0.117 ± 0.0006	0.50	0.0182 ± 0.00045	2.46
40	10.0	0.158 ± 0.0013	0.82	0.0240 ± 0.00071	2.95
50	12.5	0.195 ± 0.0029	1.47	0.0306 ± 0.00055	1.79
60	15.0	0.232 ± 0.0057	2.48	0.0362 ± 0.00084	2.31
70	17.5	0.270 ± 0.0044	1.65	0.0426 ± 0.00114	2.68

[a] D_1 = derivative response; each value is the mean ± standard deviation (n = 5).
[b] CV = coefficient of variation.

Table 2. Regression analysis of the calibration curves of Series A–E (first-derivative spectroscopy method) and of Series I–III (HPTLC method)

Series	Composition, μg/mL		Regression equation	Correlation coefficient
	LST	HCTZ		
			First-derivative spectroscopy	
A	30–70	0	$y = 0.0038x + 0.00476$[a]	0.9998
B	0	7.5–17.5	$y = 0.00244x - 0.00002$[b]	0.9992
C	30–70	12.5	$y = 0.00381x + 0.00238$[a]	0.9949
D	50	7.5–17.5	$y = 0.00244x + 0.00002$[b]	0.9989
E	30–70	7.5–17.5	$y = 0.0038x + 0.00440$[a]	0.9998
			$y = 0.00244x - 0.00018$[b]	0.9997
			HPTLC	
I	400–1200	0	$y = 4.27577x + 2441.06$[a]	0.9959
II	0	100–300	$y = 13.75696x + 719.74$[b]	0.9991
III	400–1200	100–300	$y = 4.10787x + 2541.44$[a]	0.9944
			$y = 13.91790x + 696.18$[b]	0.9979

[a] Equation for calibration curve for LST.
[b] Equation for calibration curve for HCTZ.

day and on 3 different days over a period of 1 week for each concentration of LST and HCTZ, and the results were reported in terms of percent coefficient of variation (%, CV).

Accuracy.—The accuracy of the method was determined by calculating the recoveries of LST and HCTZ by the method of standard additions. Known amounts of the drugs (LST at 0, 10, 20, 30, and 40 μg/mL and HCTZ at 0, 2.5, 5.0, 7.5, and 10 μg/mL) were added to a prequantitated sample solution (containing LST at 30 μg/mL and HCTZ at 7.5 μg/mL), and the amounts of LST and HCTZ were estimated by measuring derivative responses at the appropriate wavelength (271.6 nm for LST and 335.0 nm for HCTZ).

Linearity.—The linearity of the derivative responses for LST and HCTZ was determined at 271.6 and 335.0 nm, re-

Table 3. Summary of validation parameters for the proposed first-derivative spectroscopy and HPTLC methods

Parameter	First-derivative spectroscopy		HPTLC	
	LST	HCTZ	LST	HCTZ
Limit of detection	2.0 μg/mL	2.5 μg/mL	30 ng/spot	50 ng/spot
Linearity range	30–70 μg/mL	7.5–17.5 μg/mL	400–1200 ng/spot	100–300 ng/spot
Correlation coefficient	0.9949–0.9998	0.9989–0.9997	0.9944–0.9959	0.9979–0.9991
Precision (%, CV)[a]				
Intraday ($n = 3$)	0.49–2.99	1.30–3.15	0.57–2.72	0.94–3.98
Interday ($n = 3$)	0.77–3.71	1.33–4.20	0.67–5.00	1.88–5.56
Repeatability of measurement ($n = 7$)			0.034 (area; <0.5%)[b]	0.057 (area; <0.5%)[b]
Repeatability of sample application ($n = 7$)			1.109 (area; <3%)[b]	1.170 (area; <3%)[b]
Accuracy (%)	98.25–103.16	97.76–102.62	96.93–102.87	97.37–101.93
Specificity	Specific	Specific	Specific	Specific

[a] CV = coefficient of variation.
[b] Values in parentheses indicate corresponding prescribed limits for repeatability of measurement and sample application.

Table 4. Results for the determination of LST and HCTZ in their combined dosage forms by the proposed first-derivative spectroscopy and HPTLC methods

Method	Formulation	Labeled amount, mg/tablet LST	HCTZ	LST found ± SD, %[a] (*n* = 5)	CV, %[b]	HCTZ found ± SD, % (*n* = 5)	CV, %[b]
First-derivative spectroscopy	X	50	12.5	101.29 ± 1.93	1.91	99.64 ± 1.61	1.61
	Y	50	12.5	102.04 ± 1.75	1.71	100.93 ± 1.61	1.59
HPTLC	X	50	12.5	100.62 ± 3.09	3.07	102.25 ± 2.67	2.61
	Y	50	12.5	102.06 ± 1.95	1.91	101.23 ± 2.26	2.23

[a] SD = standard deviation.
[b] CV = coefficient of variation.

spectively, by plotting the derivative response versus the corresponding concentration of LST or HCTZ, for different series of solutions (A–E).

Application of First-Derivative Spectroscopy to the Simultaneous Determination of LST and HCTZ in Their Combined Dosage Forms

Sample preparation.—Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder equivalent to 25 mg LST (or 6.25 mg HCTZ) was transferred to a conical flask. The powder was suspended in 20 mL methanol. The suspension was sonicated for 20 min and centrifuged for 10 min at 2500–2600 rpm. The supernatant was collected in a 25 mL volumetric flask. The residues were washed with 5 mL methanol, and the washes were centrifuged as described above. The supernatant solutions were combined in a 25 mL volumetric flask and diluted to volume with methanol. An appropriate volume of the above solution was diluted further with water to obtain a final sample solution containing LST equivalent to 50 μg/mL (or HCTZ equivalent to 12.5 μg/mL).

Estimation of LST and HCTZ.—The derivative responses of the sample solution were measured at 271.6 and 335.0 nm for quantitation of LST and HCTZ, respectively. The amount of LST (or HCTZ) present in the sample solution was determined by fitting the derivative response into the equation of the line representing the calibration curve for LST (or HCTZ) and correcting for the dilution.

Experimental—High-Performance Thin-Layer Chromatography

Apparatus

A CAMAG HPTLC system consisting of a CAMAG Linomat IV semiautomatic spotting device, a CAMAG twin-trough TLC chamber, a CAMAG TLC Scanner-3, and CAMAG CATS-4 software, and a 100 μL Hamilton syringe were used.

Reagents and Materials

TLC aluminum sheets precoated with Silica Gel G60 F_{254} (layer thickness, 0.2 mm; 10 × 10 cm; E. Merck) were used after they were washed with the mobile phase, chloroform–methanol–acetone–formic acid (7.5 + 1.5 + 0.5 + 0.03, v/v).

All chemicals including chloroform, acetone (Sisco Research Laboratory Chem.), and formic acid (Fischer Scientific) were analytical-reagent grade. Other reagents and materials were the same as those described for the first-derivative spectroscopy method.

Preparation of Standard Solutions

(**a**) *LST standard solution.*—A 1 mg/mL stock solution of LST was prepared in methanol. A 1 mL aliquot of this solution was further diluted to 10 mL with methanol to obtain an LST concentration of 100 μg/mL (Solution L).

(**b**) *HCTZ standard solution.*—A 1 mg/mL stock solution of HCTZ was prepared in methanol. An appropriate volume was diluted with methanol to obtain a standard solution of

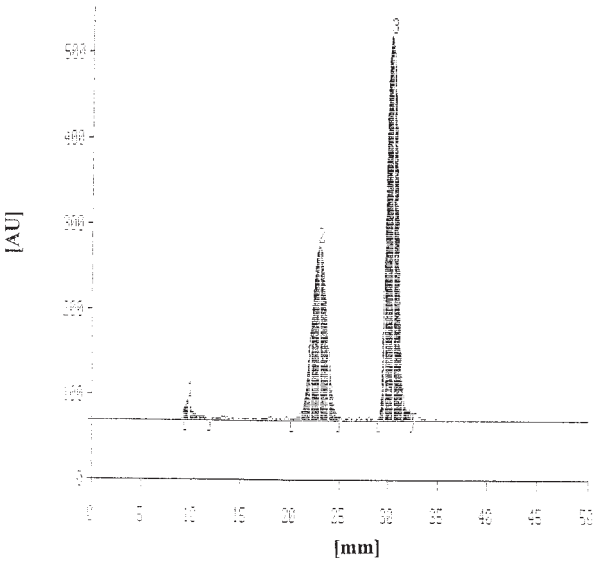

Figure 4. Chromatogram showing the separation of HCTZ (peak 2) and LST (peak 3) on a Silica Gel G60 F_{254} TLC plate.

Table 5. Calibration curve data for the determination of LST and HCTZ in the presence of each other (Series III) by the proposed HPTLC method

Series III composition, ng/spot					
LST	HCTZ	Peak area (LST)[a]	CV, %[b]	Peak area (HCTZ)[a]	CV, %[b]
400	100	4033.22 ± 101.84	2.53	2014.84 ± 029.74	1.48
600	150	5082.10 ± 124.04	2.44	2824.14 ± 103.72	3.67
800	200	6007.16 ± 097.02	1.62	3537.76 ± 122.64	3.47
1000	250	6674.16 ± 167.87	2.52	4231.34 ± 208.08	4.92
1200	300	7345.06 ± 075.60	1.03	4790.72 ± 250.77	5.23

[a] Each value is the mean ± standard deviation (n = 5).
[b] CV = coefficient of variation.

HCTZ having a concentration of 250 μg/mL. It was diluted again with methanol to obtain a standard solution of HCTZ at 25 μg/mL (Solution H).

(**c**) *Combined standard solution of LST and HCTZ.*—A combined standard solution was prepared by mixing 1 mL LST stock solution (1 mg/mL) and 1 mL HCTZ standard solution (250 μg/mL) in a 10 mL volumetric flask and diluting to volume with methanol (Solution LH).

Chromatographic Conditions

Chromatographic estimations were performed by using prewashed TLC plates under the following conditions: mobile phase, chloroform–methanol–acetone–formic acid (7.5 + 1.5 + 0.5 + 0.03, v/v); chamber saturation time, 45 min; temperature, 25 ± 3°C; migration distance, 35 mm; wavelength, 254 nm; and slit dimension, 4 × 0.2 mm. The sample was applied by using the following spotting parameters: bandwidth, 3 mm; space between 2 bands, 5 mm; and spraying rate, 12 s/μL.

Chromatographic Separation

Appropriate volumes (μL) of standard solutions (Solutions L, H, and LH) were applied on a prewashed TLC plate under a nitrogen stream by using a semiautomatic spotter. The plate was dried and developed with the mobile phase in a twin-trough chamber previously saturated with 20 mL mobile phase for 45 min. The plate was removed from the chamber and dried under an infrared lamp. Photometric measurements were made at 254 nm by using the reflectance/absorbance mode of the CAMAG TLC Scanner-3 with CATS-4 software incorporating the track optimization option.

Calibration Curves for LST and HCTZ

Series I.—The calibration curve for the LST standard (400–1200 ng/spot) was obtained by applying 4, 6, 8, 10, and 12 μL Solution L on a prewashed TLC plate by using a semiautomatic spotter under a nitrogen stream. The TLC plate was developed and analyzed as described under *Chromatographic Separation*. The calibration curve was prepared by plotting peak area versus concentration of LST (ng/spot) corresponding to each spot.

Series II.—The calibration curve for the HCTZ standard (100–300 ng/spot) was prepared by applying 4, 6, 8, 10, and 12 μL Solution H on a prewashed TLC plate and developing and analyzing the plate as described for Series I.

Series III.—The calibration curves for LST (400–1200 ng/spot) and HCTZ (100–300 ng/spot) were prepared by applying 4, 6, 8, 10, and 12 μL solution LH on a prewashed TLC plate and developing and analyzing the plate as described for Series I or II.

Method Validation

Linearity.—The linearity of the responses (peak area) for LST and HCTZ were determined for concentration ranges of 400–1200 and 100–300 ng/spot, respectively.

Accuracy.—The accuracy of an analysis in terms of systematic error was determined at 3 different concentration levels of LST (400, 800, and 1200 ng/spot) and HCTZ (100, 200, and 300 ng/spot).

Precision.—(*1*) The repeatability of the measurement of peak area and peak height was determined as follows: the combined standard solution (8 μL, Solution LH) was spotted on a TLC plate, and the plate was developed as described above. The resolved spots were scanned 7 times without changing position of the plate, and the % CVof the measurements was computed. (*2*) The repeatability of the sample application was assessed by applying the combined standard solution (8 μL, Solution LH) 7 times on a TLC plate with the semiautomatic spotter, and the plate was developed and analyzed as described above. The % CV was calculated for peak height and area of the developed spots.

The intraday and interday precision were determined for LST and HCTZ by estimating the corresponding responses at 3 times on the same day and on 3 different days over a period of 1 week for each concentration of LST and HCTZ, and the results were reported in terms of % CV of the peak area.

Application of HPTLC to the Simultaneous Determination of LST and HCTZ in Their Combined Dosage Forms

Sample preparation.—A combined-tablet solution, containing LST (equivalent to 1 mg/mL) and HCTZ (equivalent to 250 μg/mL), was prepared in methanol as described in the

Table 6. Comparison of proposed first-derivative spectroscopy (FDS) and HPTLC methods

	Assay result for LST, %		Assay result for HCTZ, %	
Formulation	HPTLC	FDS	HPTLC	FDS
X	103.91	104.76	98.46	97.67
	95.48	100.55	103.73	100.95
	98.83	101.60	106.23	97.67
	102.95	100.55	100.47	100.95
	101.95	98.97	102.38	100.95
	P ($T^a \geq t$) 2-tailed	0.687	*P* ($T^a \geq t$) 2-tailed	0.172
Y	105.34	104.52	104.50	102.24
	99.31	103.48	101.63	102.24
	101.64	101.88	97.67	98.96
	101.49	100.30	100.17	102.24
	102.52	100.04	102.17	98.96
	P ($T^a \geq t$) 2-tailed	0.989	*P* ($T^a \geq t$) 2-tailed	0.786

[a] T = T CRITICAL 2-tailed (table value) = 2.306.

first-derivative spectroscopy method. An appropriate volume of this solution was suitably diluted with methanol to obtain a solution containing LST equivalent to 100 μg/mL (or HCTZ equivalent to 25 μg/mL).

Estimation of LST and HCTZ.—An 8 μL aliquot of sample solution was applied to a TLC plate and analyzed as described above. The amounts of LST and HCTZ present in the sample solution were determined from the calibration curves for LST and HCTZ, respectively.

Results and Discussion

First-Derivative Spectroscopy Method

The technique of derivative spectroscopy may be used with minimum error for the quantitation of one analyte whose peak is obscured by a large overlapping peak of another analyte. Derivative spectroscopy is used to eliminate background absorption due to formulation excipients (20–24).

The zero-order spectra of standard solutions of LST (50 μg/mL) and HCTZ (12.5 μg/mL) were found to be similar in nature and overlapping (Figure 1). It was observed that LST and HCTZ contribute significantly at their corresponding λ_{max} values for absorbance. Therefore, it was thought that a derivative graphical method could be used to estimate LST and HCTZ in the presence of each other.

The first-derivative spectra (D_1) of LST and HCTZ offered an advantage for the simultaneous determination of LST and HCTZ by having widely separated ZCPs. A D_1 spectrum of LST has zero absorbance in the 315–400 nm region, and HCTZ offers 5 ZCPs (at 231.4, 246.2, 271.6, 280.8, and 314 nm; Figure 2). Of these ZCPs of HCTZ, LST gives the highest derivative response at 271.6 nm. Therefore, 271.6 nm was selected for the quantitation of LST because it provided the highest sensitivity to the method.

In the region of the zero-derivative response of LST, at 335.0 nm, HCTZ shows a higher response. Therefore, 335.0 nm was considered the characteristic wavelength for quantitation of HCTZ. Moreover, the derivative response at 335.0 nm increases with increasing concentrations of HCTZ in Series B, D, and E (Figure 3).

It was also observed that with the increase in LST concentration, the derivative response at 271.6 nm increased. The responses for LST in Series A, C, and E were found to be linear in the range of 30–70 μg/mL, with correlation coefficients 0.9998, 0.9949, and 0.9998, respectively.

Similarly, the derivative responses for HCTZ at 335 nm were linear in the range of 7.5–17.5 μg/mL, with correlation coefficients of 0.9992, 0.9989, and 0.9997 in the case of Series B, D, and E, respectively. Table 1 shows representative data indicating derivative responses for LST and HCTZ, with respect to concentration, in the presence of each other (Series E).

The limits of detection for LST and HCTZ were 2.0 and 2.5 μg/mL, respectively.

Regression analysis.—Regression analysis of calibration curve data for Series A, C, and E (for LST) and Series B, D, and E (for HCTZ) produced no difference in the equations obtained for the calibration curves. This result indicates that there is no interference from HCTZ in the determination of LST and vice versa (Table 2). It is recommended that the Series E design be used for the preparation of calibration curves for LST as well as HCTZ.

Validation.—The average recoveries (± CV) of LST and HCTZ were 100.73 ± 2.122 and 100.54 ± 1.741%, respectively, which are satisfactory.

The intraday and interday precision for LST and HCTZ were determined for Series A, C, and E and Series B, D, and E, respectively. The method was validated in terms of limit of de-

tection, limit of quantitation, linearity range, and specificity. The validation parameters are summarized in Table 3.

Assay results for combined dosage forms.—The proposed first-derivative spectroscopy method was applied to the determination of LST and HCTZ in their combined dosage forms (tablets). The results obtained for LST and HCTZ in the combined formulations were comparable with the corresponding labeled amounts (Table 4).

HPTLC Method

Chromatographic conditions.—Various solvent systems, i.e., chloroform–methanol, chloroform–methanol–ethyl acetate, and chloroform–methanol–ammonia solution, in different proportions, were tried to resolve LST and HCTZ spots on the Silica Gel G60 F_{254} TLC plate. The combination of methanol and chloroform provided optimum polarity for the proper migration of LST and HCTZ on the plate. It was observed that acetone favored movement of HCTZ and could help to achieve the desired resolution of LST and HCTZ. However, the corresponding peaks were not sharp, and tailing was observed in the case of the LST peak. This was due to the interaction of the acidic tetrazol group (pK_a of LST, 2.36 and 5.54) in LST and the silanol groups of the silica on the TLC plate (10). To suppress this interaction and decrease the tailing effect, various modifiers, i.e., ammonia solution, triethylamine, acetic acid, and formic acid, were tried. Of these, formic acid gave the best results. Thus, the mobile phase chloroform– methanol–acetone–formic acid (7.5 + 1.5 + 0.5 + 0.03, v/v) gave 2 well-separated, sharp, and symmetrical peaks for LST and HCTZ at Rf values of 0.61 ± 0.02 and 0.41 ± 0.02, respectively (Figure 4).

Prewashing the plate with mobile phase and saturating the chamber (45 min) with mobile phase helped to achieve better resolution and sharpness of the LST and HCTZ peaks.

Photometric measurements were made in the reflectance/absorbance mode with the CAMAG TLC Scanner-3 at 254 nm, which is suitable for all UV-absorbing compounds. LST and HCTZ were determined by using the corresponding peak areas and the calibration curve equations obtained from Series III.

Linearity of LST and HCTZ.—The calibration curve for LST (Series I) was obtained by plotting the peak area of LST versus the concentration of LST over the range of 400–1200 ng/spot. The correlation coefficient was 0.9959, and the CV was in the range of 0.94–3.54%.

The linearity range for HCTZ (Series II) was 100–300 ng/spot, with a correlation coefficient of 0.9991 and a CV ranging from 2.67 to 4.55%.

Similarly, the calibration curves for LST and HCTZ were prepared for a mixture of LST and HCTZ (Series III; Table 5). The peak areas for LST in the range of 400–1200 ng/spot and for HCTZ in the range of 100–300 ng/spot were linear, with correlation coefficients of 0.9944 and 0.9979, respectively.

These results indicate that there is no interference from LST in the determination of HCTZ and vice versa. This absence of interference is also reflected in the regression analysis of different calibration curves (close values of slope and intercept) obtained for LST and HCTZ from Series I– III (Table 2). The Series III design is recommended for use as a model for the preparation of calibration curves for LST and HCTZ.

The limits of detection for LST and HCTZ were 30 and 50 ng/spot, respectively.

Accuracy.—The accuracy was determined at 3 different concentration levels, 400, 800, and 1200 ng/spot for LST and 100, 200, and 300 ng/spot for HCTZ. Accuracy was expressed in terms of average recoveries (± CV), which were 99.673 ± 3.016% for LST and 99.147 ± 2.462% for HCTZ.

Precision.—The repeatability of the measurement of peak area and peak height based on 7 measurements of the same spot, and the repeatability of the sample application, based on 7 applications, indicated that the corresponding % CV values are lower than the prescribed limits; thus, the measurements of response and sample application are reliable.

The intraday and interday CV values for LST determination ranged from 0.57 to 2.72% and from 0.67 to 5.0%, respectively.

The intraday and interday CV values for HCTZ determination ranged from 0.94 to 3.98% and from 1.88 to 5.56%, respectively.

Specificity.—It was observed that excipients present in the formulation did not interfere with the resolution and sharpness of the peaks of LST (Rf = 0.61 ± 0.02) and HCTZ (Rf = 0.41 ± 0.02). Both peaks were sharp and well resolved. These results indicate that the method is specific for LST and HCTZ.

The validation parameters for the HPTLC method are summarized in Table 3.

Assay results for combined dosage forms.—The proposed HPTLC method was applied to the estimation of LST and HCTZ in their combined dosage forms (tablets). The results obtained indicate that the LST and HCTZ amounts found in the dosage forms are comparable with the labeled amounts (or label claims; Table 4).

Thus, the proposed HPTLC method was found to be simple, specific, precise, and accurate for the estimation of LST and HCTZ in their combined dosage forms.

Comparison of Proposed Methods

The assay results for LST and HCTZ in combined dosage forms, obtained by using the first-derivative spectroscopy and HPTLC methods, were compared by applying the 2-tailed Student's *t*-test at the 95% ($P = 0.05$) confidence level. Because the calculated *t*-values were less than the table *t*-value (i.e., T CRITICAL), there is no significant difference in the content of LST and HCTZ determined by the HPTLC method and the first-derivative spectroscopy method (Table 6). This, in turn, indicates that both methods are precise and accurate and can be used successfully for routine quality control of combined dosage forms of LST and HCTZ.

Conclusions

Two methods based on different analytical techniques, first-derivative spectroscopy and HPTLC, were developed for the determination of LST and HCTZ in the presence of each other. Both methods were validated and were found to be simple, rapid, sensitive, specific, accurate, and precise. The meth-

ods were successfully used to estimate the amounts of LST and HCTZ present in 2 marketed tablet formulations containing LST and HCTZ. The assay values for LST in formulations X and Y were 101.29 and 102.04%, respectively, and the corresponding HCTZ values were 100.62 and 102.06%. The statistical comparison of the assay results obtained for LST and HCTZ in the tablet formulations by using these methods indicated no significant difference. Because they are fast, simple, specific, precise, and accurate, both methods can be used for routine determinations of LST and HCTZ in their combined dosage formulations.

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