

Analytical Profiling of Indapamide and Losartan Potassium: UV–Visible, FTIR, and Chromatographic Characterization for Simultaneous Analysis

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Abstract:

Background: Simultaneous analysis of drug combinations requires prior understanding of the individual physicochemical and spectroscopic behavior of each analyte and systematic optimization of chromatographic selectivity. Indapamide (IND), a thiazide-like diuretic, and losartan potassium (LOS), an angiotensin II receptor blocker, differ markedly in aqueous solubility, lipophilicity and UV absorption behavior. **Objective:** The present work profiled IND and LOS by preliminary physicochemical tests, FTIR, UV–visible spectrophotometry and RP-HPLC to establish analytical conditions suitable for simultaneous determination. **Methods:** Melting point and solubility were evaluated, FTIR spectra were interpreted for characteristic functional groups, and methanolic UV spectra were scanned from 200–400 nm. Chromatographic screening was performed on a C18 column (250 × 4.6 mm, 5 µm) using seven mobile-phase trials. **Results:** IND showed a melting point of 163–165°C and LOS 262–264°C. Characteristic FTIR bands supported the identity of both analytes. UV maxima were approximately 236 nm for IND and 206 nm for LOS; an overlay supported 214 nm as the common HPLC detection wavelength. The optimized chromatographic system consisted of phosphate buffer pH 3.0): acetonitrile (40:60, v/v) at 1.0 mL/min. IND and LOS eluted at approximately 3.74 and 6.98 min, respectively, with a resolution of 11.22. Calibration responses were linear over 0.75–7.5 µg/mL for IND and 25–250 µg/mL for LOS, with R² values of 0.9997 and 0.9996. **Conclusion:** Complementary UV, FTIR and chromatographic profiling provided a rational basis for simultaneous analysis of IND and LOS and established a selective RP-HPLC condition for their quantitative determination.

Keywords: Indapamide; Losartan potassium; UV–visible spectroscopy; FTIR; RP-HPLC

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1. INTRODUCTION

Hypertension is commonly managed with drug combinations that act through complementary mechanisms. Indapamide is a thiazide-like diuretic, whereas losartan potassium is an angiotensin II receptor blocker. Their combined use has pharmacological rationale because diuresis reduces sodium and water retention while angiotensin II receptor blockade reduces vasoconstriction and aldosterone-mediated fluid retention^{1,2}.

From an analytical perspective, simultaneous determination of two chemically different drug substances can be challenging. Differences in ionization, polarity, solubility and spectral absorption influence sample preparation, wavelength selection, retention and peak shape. Consequently, preliminary analytical profiling is useful before formal quantitative method validation^{3,4}.

The literature summarized in the source study showed numerous individual analytical procedures for LOS and IND, including UV spectrophotometry, HPLC/RP-HPLC, HPTLC and LC-MS/MS. However, the reviewed set did not identify a reported simultaneous stability-indicating HPLC method specifically for the IND-LOS pair. This provided the analytical basis for systematic development of a combined chromatographic procedure⁵⁻¹⁵.

Current analytical-development principles also emphasize understanding the purpose of the procedure and the variables affecting performance. ICH Q14 describes science- and risk-based analytical procedure development, while ICH Q2(R2) provides the contemporary framework for demonstrating analytical performance characteristics^{16,17}.

Aim

To establish an analytical profile of indapamide and losartan potassium using physicochemical characterization, FTIR spectroscopy, UV-visible spectrophotometry and RP-HPLC, and to use the combined information to select chromatographic conditions suitable for simultaneous analysis.

Objectives

- To compare the physicochemical characteristics and solvent behavior of IND and LOS.
- To confirm characteristic functional groups by FTIR spectroscopy.
- To determine the UV absorption maxima of both drugs and select a common analytical wavelength.
- To characterize retention, peak symmetry, efficiency, resolution, specificity and calibration response.

1. DRUG AND ANALYTICAL PROFILE

Table 1. Comparative physicochemical profile of indapamide and losartan potassium

Parameter	Indapamide	Losartan potassium
Therapeutic class	Thiazide-like diuretic	Angiotensin II receptor blocker
Molecular formula	C ₁₆ H ₁₆ ClN ₃ O ₃ S	C ₂₂ H ₂₂ ClN ₆ O
pKa	8.8	~3.1 (tetrazole group)

Log P	2.2	4.0
Reported melting point	160–162°C	263–265°C
Observed melting point	163–165°C	262–264°C
Water solubility	Practically insoluble	Freely soluble
Methanol	Freely soluble	Freely soluble
Acetonitrile	Soluble	Soluble/sparingly-to-moderately soluble in preliminary screening

The two analytes exhibit contrasting aqueous solubility and ionizable functional groups. IND contains sulfonamide and amide functionalities, whereas LOS contains an imidazole/tetrazole-containing structure and is supplied as the potassium salt. These differences were important during selection of extraction solvent, aqueous-phase pH and organic modifier^{3,5}.

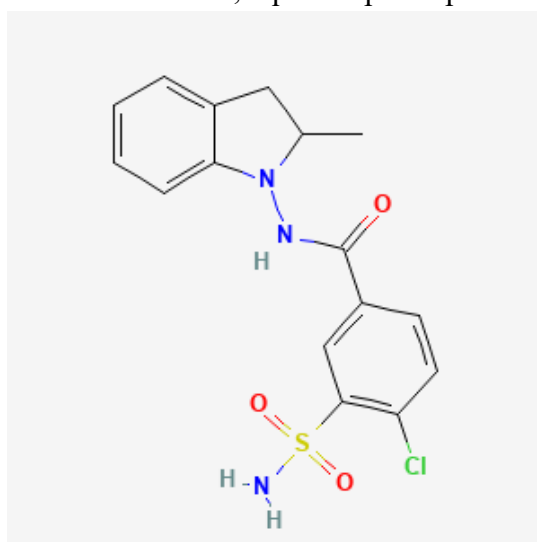


Figure 1A. Chemical structure of indapamide reproduced from the experimental presentation.

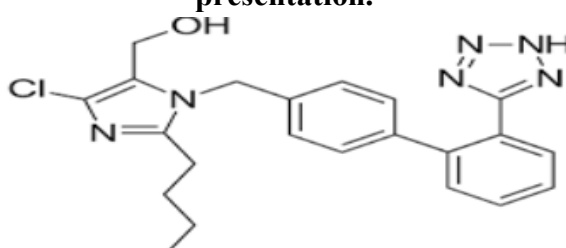


Figure 1B. Chemical structure of losartan reproduced from the experimental presentation.

2. MATERIALS AND METHODS

Chemicals and reagents

Indapamide and losartan potassium reference materials were used for analytical development. HPLC-grade methanol and acetonitrile were used as organic solvents. Orthophosphoric acid, potassium dihydrogen phosphate and disodium hydrogen phosphate of analytical grade were used for aqueous-phase preparation and pH adjustment. HPLC-grade water was used for chromatographic work¹⁹⁻²³.

Instrumentation**Table 2. Major instruments used in the analytical study**

Instrument	Model/manufacturer	Purpose
HPLC system	Shimadzu LC-20 AD	Chromatographic development and quantitative analysis
UV–visible spectrophotometer	Shimadzu UV-1780	200–400 nm spectral scanning
FTIR spectrometer	Bruker Alpha-II	Functional-group/identity profiling
Analytical balance	Shimadzu AUW 220D	Accurate weighing of standards and samples
Ultrasonicator	Trans-o-sonic	Dissolution/extraction
Digital pH meter	MAC/SR No. 1706	Buffer pH adjustment

3. RESULTS**Preliminary solubility and melting-point assessment**

Solubility of both analytes was screened in water, methanol, ethanol and acetonitrile. This preliminary experiment aimed to identify a solvent that produces stable stock solutions while remaining compatible with chromatographic development. Melting points were recorded as an additional identity-oriented physical parameter and compared with the ranges used in the source presentation.

FTIR procedure

FTIR spectra of the drug substances were acquired using the Bruker Alpha-II instrument. The spectra were examined for the major absorption bands expected from the functional groups present in each molecule. Interpretation focused on the principal bands used in the experimental dataset rather than assigning every minor spectral feature.

UV–visible procedure

Standard solutions of IND and LOS were prepared in methanol and scanned between 200 and 400 nm. Individual absorption maxima were identified and the spectra were compared to determine a wavelength region that could provide simultaneous chromatographic detectability. The experimental overlay ultimately supported selection of 214 nm for RP-HPLC monitoring.

FTIR CHARACTERIZATION

The FTIR spectrum of IND showed a broad/characteristic N–H stretching region at 3404.25 cm^{-1} , aliphatic C–H stretching near 2954.85 cm^{-1} , a strong carbonyl band at 1679.32 cm^{-1} , aromatic C=C vibration at 1582.47 cm^{-1} , C–N-related bands at 1310.92 and 1278.28 cm^{-1} , S=O stretching at 1186.65 cm^{-1} and a C–Cl-associated band at 780.92 cm^{-1} . Together, these signals supported the expected functional-group profile of indapamide.

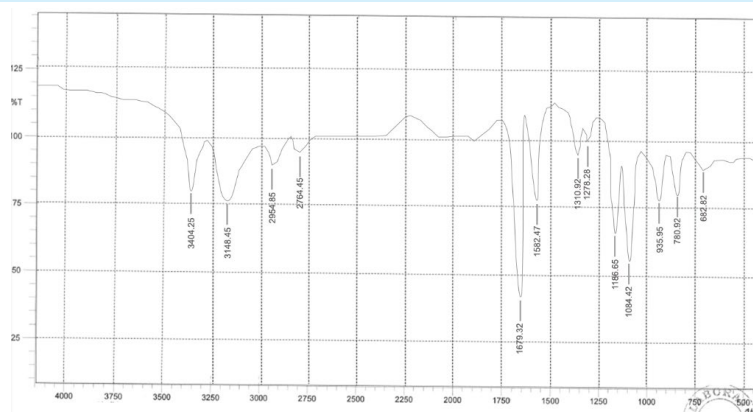


Figure 2. FTIR spectrum of indapamide used for functional-group characterization.

Table 3. Major FTIR bands of indapamide

Observed wavenumber (cm ⁻¹)	Assignment
3404.25	N–H stretching
2954.85	C–H stretching
1679.32	C=O stretching
1582.47	Aromatic C=C stretching
1310.92, 1278.28	C–N vibrations
1186.65	S=O stretching
780.92	C–Cl vibration

FTIR CHARACTERIZATION OF LOSARTAN POTASSIUM

The LOS FTIR spectrum showed bands at approximately 3348.65 and 3208.98 cm⁻¹ in the O–H/N–H stretching region, a band at 2872.50 cm⁻¹ attributed in the experimental interpretation to N–H/C–H-related vibration, C=N/aromatic heterocyclic vibrations at 1488.70 and 1581.60 cm⁻¹, a tetrazole-associated N=N region at 1360.92 cm⁻¹, C–O-related bands at 1231.82 and 1106.32 cm⁻¹ and aromatic out-of-plane bending bands at 701.92, 778.06 and 888.80 cm⁻¹.

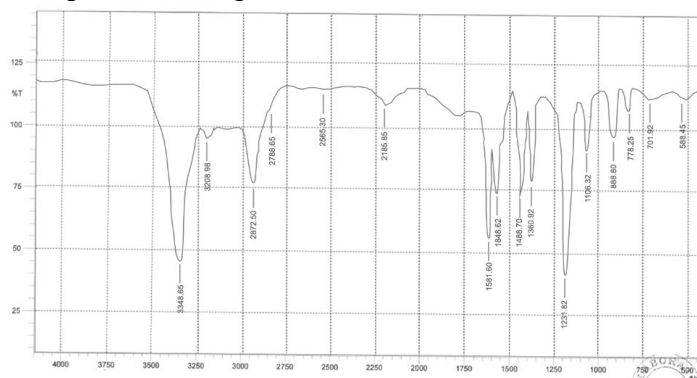


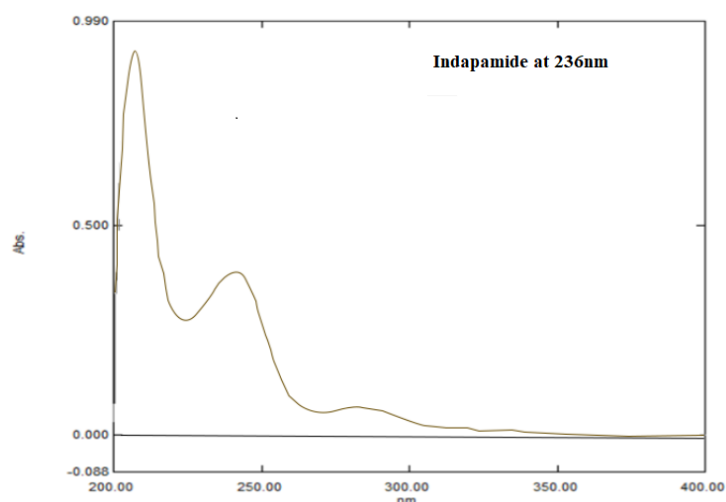
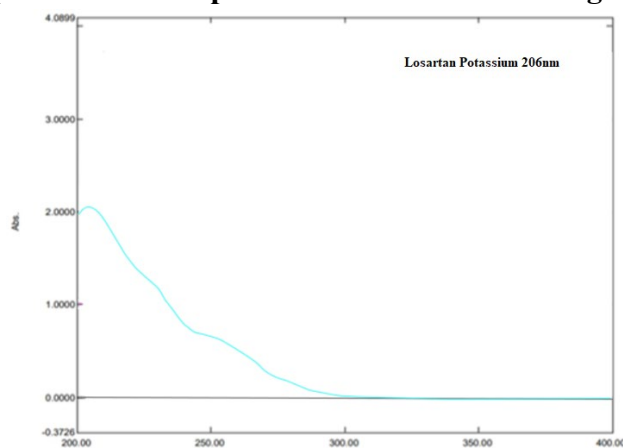
Figure 3. FTIR spectrum of losartan potassium used for functional-group characterization.

Table 4. Major FTIR bands of losartan potassium

Observed wavenumber (cm ⁻¹)	Experimental assignment
3348.65, 3208.98	O–H/N–H stretching region
2872.50	N–H/C–H-related stretching
1581.60, 1488.70	C=N/aromatic heterocyclic vibration
1360.92	Tetrazole N=N region
1231.82, 1106.32	C–O-related vibration
701.92, 778.06, 888.80	Aromatic C–H bending region

UV-VISIBLE SPECTRAL PROFILING

Methanolic UV scanning demonstrated clearly different absorption behavior for the two analytes. IND exhibited a prominent absorption maximum at approximately 236 nm, whereas LOS showed strong absorption around 205–206 nm. This spectral separation is analytically useful for individual characterization but requires compromise when a single HPLC detector wavelength is used for simultaneous measurement.

Figure 4A. UV spectrum of indapamide in methanol showing λ_{max} near 236 nm.Figure 4B. UV spectrum of losartan potassium in methanol showing λ_{max} near 206 nm.

The overlay spectrum obtained during method development was therefore evaluated to identify a common wavelength with adequate response from both drugs. A wavelength of 214

nm was selected for chromatographic analysis. This choice did not represent the individual λ_{max} of either analyte; rather, it was an experimentally selected common wavelength intended to provide simultaneous detectability.



Figure 5. HPLC system used during chromatographic method development and simultaneous analysis.

PREPARATION OF STANDARD AND SYNTHETIC-MIXTURE SOLUTIONS

Standard stock solutions

For IND, 10 mg was transferred to a 100 mL volumetric flask, dissolved in methanol and diluted to volume to obtain 100 $\mu\text{g/mL}$. For LOS, 100 mg was similarly dissolved and diluted to 100 mL with methanol to obtain 1000 $\mu\text{g/mL}$. A mixed working solution was prepared by transferring 0.3 mL of the IND stock and 1.0 mL of the LOS stock to a 10 mL volumetric flask and diluting with methanol, providing 3 $\mu\text{g/mL}$ IND and 100 $\mu\text{g/mL}$ LOS.

Calibration levels

Further dilutions generated IND calibration levels of 0.75, 1.5, 3.0, 4.5, 6.0 and 7.5 $\mu\text{g/mL}$ and LOS calibration levels of 25, 50, 100, 150, 200 and 250 $\mu\text{g/mL}$. The concentration ranges preserved the approximate dose-ratio relationship used in the source experimental design.

Synthetic mixture

Table 5. Composition of the synthetic pharmaceutical mixture

Component	Amount per 100 mg mixture
Indapamide	1.5 mg
Losartan potassium	50 mg
Microcrystalline cellulose	37.5 mg
Lactose monohydrate	8.0 mg
Magnesium stearate	2.0 mg
Talc	1.0 mg

Sample preparation

Powder equivalent to 1.5 mg IND and 50 mg LOS was transferred to a 10 mL volumetric flask, methanol was added, and the mixture was sonicated for 10 min. The volume was completed with methanol to obtain nominal concentrations of 150 $\mu\text{g/mL}$ IND and 5000 $\mu\text{g/mL}$ LOS.

LOS. A 0.1 mL aliquot was diluted to 10 mL with methanol, producing final assay concentrations of 1.5 µg/mL IND and 50 µg/mL LOS.

CHROMATOGRAPHIC METHOD DEVELOPMENT

A reversed-phase C18 column was selected because the two analytes showed moderate hydrophobic character and because C18 phases were commonly represented in the literature reviewed in the source study. Mobile-phase development focused on phosphate-buffered aqueous phases combined with acetonitrile and/or methanol. Seven trials were performed, with peak symmetry, splitting and simultaneous resolution used as practical selection criteria.

Table 6. Mobile-phase screening during RP-HPLC optimization

Trial	Mobile phase	Observation
1	Phosphate buffer pH 3.5:ACN (50:50)	Asymmetrical peaks
2	Phosphate buffer pH 3.5:methanol (40:60)	Peak splitting
3	Water:methanol (60:40)	Only one drug peak with splitting
4	Phosphate buffer pH 3.0:ACN (45:55)	Asymmetrical peak
5	Phosphate buffer pH 2.5:methanol (55:45)	Peak splitting
6	Phosphate buffer pH 3.0:methanol:ACN (40:35:25)	Peak splitting
7	Phosphate buffer pH 3.0:ACN (40:60)	Symmetrical, resolved peaks; selected

<Chromatogram>

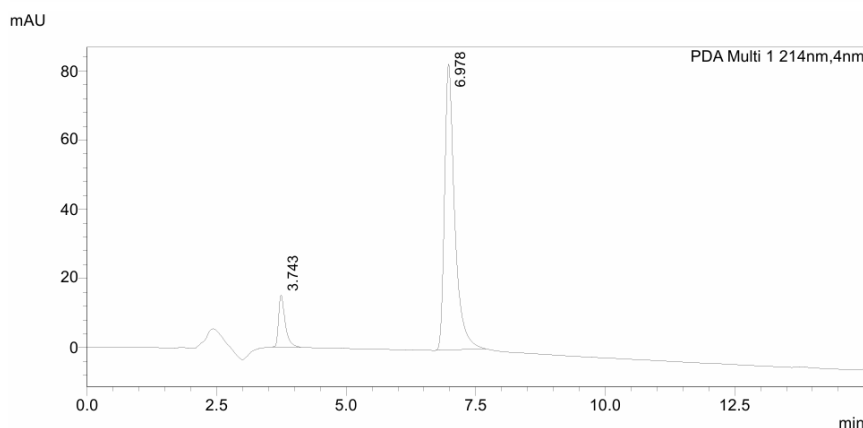


Figure 6. Chromatogram of the selected Trial 7 condition showing satisfactory simultaneous separation.

The selected mobile phase was phosphate buffer pH 3.0:acetonitrile (40:60, v/v). The final flow rate was 1.0 mL/min, injection volume 10 µL, detection wavelength 214 nm and run time 15 min.

CHROMATOGRAPHIC CHARACTERIZATION AND SPECIFICITY

Under the optimized conditions, IND eluted at approximately 3.74 min and LOS at approximately 6.98 min. The retention difference produced a reported resolution of 11.22 ± 0.12 , indicating clear separation of the two analyte peaks in the experimental system. System

suitability also showed high theoretical-plate counts and acceptable tailing factors for both compounds.

Table 7. System-suitability characteristics of IND and LOS

Parameter	Indapamide	Losartan potassium
Retention time (min)	3.74 ± 0.05	6.98 ± 0.06
Retention-time %RSD	1.34	0.98
Theoretical plates	25,193 ± 289.49	44,165 ± 520.40
Plate-count %RSD	1.15	1.18
Tailing factor	1.42 ± 0.02	1.46 ± 0.01
Tailing %RSD	1.08	1.07
Resolution	—	11.22 ± 0.12

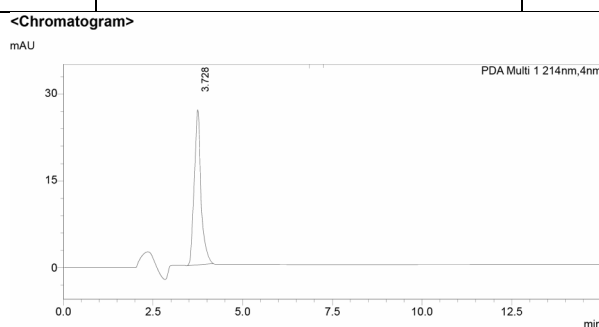


Figure 7A. Representative RP-HPLC chromatogram of indapamide.

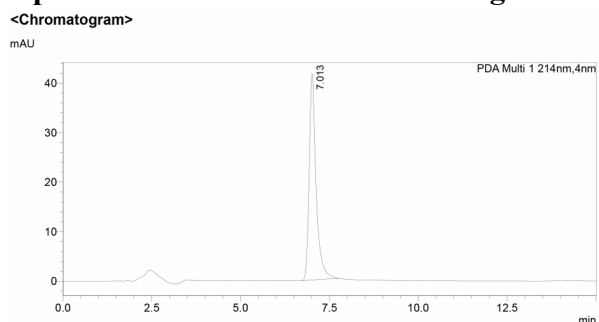


Figure 7B. Representative RP-HPLC chromatogram of losartan potassium.

Specificity was evaluated by comparing individual analyte injections, the mixed standard/sample response and blank behavior. The source experiment reported no interfering response at the analyte retention times, and peak-purity assessment was used as additional chromatographic evidence.

10. SIMULTANEOUS RESPONSE, LINEARITY AND CALIBRATION

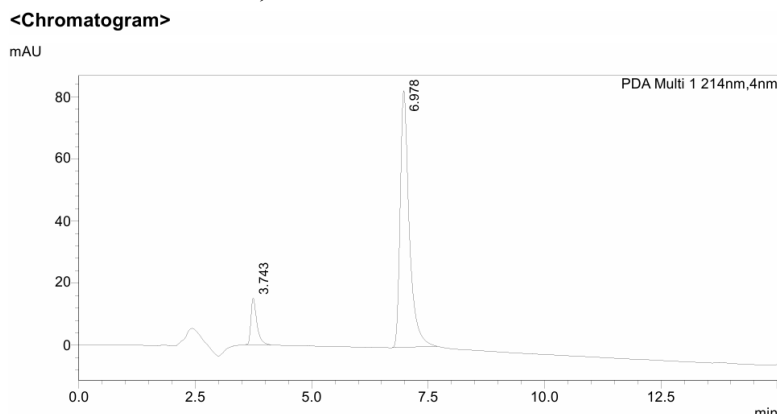


Figure 8. Representative chromatogram of the IND–LOS mixture under optimized RP-HPLC conditions.

Calibration response was evaluated across six concentration levels for each drug. IND was linear from 0.75 to 7.5 $\mu\text{g/mL}$ with $y = 40748x + 599.04$ and $R^2 = 0.9997$. LOS was linear from 25 to 250 $\mu\text{g/mL}$ with $y = 10214x + 16280$ and $R^2 = 0.9996$. These data demonstrate proportional detector response across the concentration ranges used for simultaneous analysis.

Table 8. Calibration data for simultaneous analysis

Level	IND conc. ($\mu\text{g/mL}$)	IND area $\pm$ SD	LOS conc. ($\mu\text{g/mL}$)	LOS area $\pm$ SD
1	0.75	31,854.50 $\pm$ 425.83	25	274,764.64 $\pm$ 4,307.63
2	1.50	60,319.28 $\pm$ 936.92	50	540,442.62 $\pm$ 5,181.03
3	3.00	125,373.75 $\pm$ 2,105.28	100	1,020,872.31 $\pm$ 16,368.16
4	4.50	181,157.38 $\pm$ 2,736.18	150	1,552,173.52 $\pm$ 30,582.62
5	6.00	245,608.64 $\pm$ 4,532.51	200	2,034,074.91 $\pm$ 36,811.06
6	7.50	306,683.14 $\pm$ 4,753.47	250	2,591,280.50 $\pm$ 32,606.62

4. DISCUSSION

The analytical profiling strategy demonstrated why multiple complementary techniques were useful before simultaneous chromatographic quantification. Melting point and solubility provided basic physical information; FTIR supported identity through functional-group fingerprints; UV spectroscopy established individual absorption behavior; and RP-HPLC provided separation and quantitative response. No single technique alone provided all the information required for a rational simultaneous method.

The pronounced difference between the UV maxima of IND (~ 236 nm) and LOS (~ 206 nm) illustrates a practical challenge in simultaneous UV-based measurement. Selecting 214 nm for HPLC was an experimental compromise that preserved detectable response for both analytes. In

a PDA-based chromatographic system, the wavelength can be chosen according to the analytical objective rather than requiring it to coincide exactly with either individual λ_{max} .

Mobile-phase screening showed that chromatographic performance was sensitive to both organic modifier and aqueous-phase pH. Methanol-containing conditions repeatedly produced splitting in the experimental trials, while several acetonitrile conditions improved peak shape. The selected pH 3.0 phosphate buffer:acetonitrile 40:60 system produced symmetrical and well-resolved peaks. This empirical optimization is consistent with the analytical-development concept described in ICH Q14, which emphasizes understanding procedure parameters and their relationship to performance.

The final chromatographic profile showed IND and LOS retention at 3.74 and 6.98 min with resolution of 11.22. Calibration coefficients above 0.999 for both drugs further supported the suitability of the selected detector response and concentration ranges. ICH Q2(R2) identifies specificity/selectivity, accuracy and precision among the central performance characteristics for quantitative analytical procedures and recognizes that multiple characteristics may be supported by the same experimental dataset.

A limitation of the present article is that it is based on the synthetic mixture and experimental conditions documented in the source study. It should not be interpreted as independent evidence for every marketed formulation or manufacturing matrix. Transfer to a different dosage form, excipient system, instrument, or column lot should be supported by appropriate verification and, where necessary, additional validation.

5. CONCLUSION

A structured analytical profile of indapamide and losartan potassium was established using physicochemical characterization, FTIR, UV-visible spectroscopy and RP-HPLC. The two drugs showed distinct solubility and UV absorption behavior but could be monitored simultaneously after selection of a common chromatographic wavelength. FTIR spectra provided characteristic functional-group patterns for both analytes. Systematic mobile-phase screening identified phosphate buffer pH 3.0:acetonitrile (40:60, v/v) as the most satisfactory condition among the seven evaluated trials. The optimized system produced well-separated IND and LOS peaks at approximately 3.74 and 6.98 min, respectively, with high chromatographic resolution and linear detector response across the investigated ranges. The combined profiling approach therefore provided a scientifically coherent basis for subsequent simultaneous quantitative and stability-indicating analysis.

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Data availability: The analytical data reported in this manuscript were derived from the experimental presentation supplied by the authors.

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