Supporting Information

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Novel analytical method development and validation for simultaneous estimation of curcumin, ascorbic acid and salicylic acid in bulk and its pharmaceutical formulation by RP-HPLC

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Sr. no	Active constituent	Reported method	Method Specification/Summary
1.	Curcumin	HPLC	• Column: Diamonsil C18 analytical column (4.6 x 100 mm, 5 micron) • Mobile phase: acetonitrile-5% acetic acid (75:25, v/v) • Flow rate: 1.0 mL/min • linearity range :1-500 ng/mL
2.	Curcuminoids	HPLC	• Column: Alltima C18 column • Mobile Phase: acetonitrile and 2% v/v acetic acid (40:60, v/v) • Flow rate: 2.0 mL/min • Column temp.: 33 °C, UV detection: 425 nm • Curcumin: 10–60 µg/mL (99.16–101.75%), Desmethoxycurcumin: 4–24 µg/mL (99.50–101.01 %), Bisdesmethoxycurcumin: 0.5–3.0 µg/mL (99.67–101.92 %)
3.	Curcumin	HPLC	• Column: C18 • Gradient mobile phase system: acetonitrile–tetrahydrofuran–water containing 0.1% formic acid • Detection Wavelength: 425 nm
4.	Curcumin	UV-visible	• Detection Wavelength: 429 nm • Solvent system: pH 7.4 phosphate buffer and ethanol in mixture (1:1). • Concentration range: 2-10 µg/ml • Correlation coefficient: 0.998 • Accuracy: 99.79% and 100.27%
5.	Curcumin	UV-visible	• Detection Wavelength: 421nm • Solvent: Methanol • Linearity range: 5-25µg/ml • Correlation coefficient: 0.9997 • LOD: 0.4 µg/ml • LOQ:1.21µg/ml
6.	Curcumin	UV and HPLC	Curcuma longa (Turmeric) is used successfully in Ayurvedic formulations from ancient times. It is a rich source of bioactive phytoconstituents like curcuminoids, turmerone and many more. Curcuminoids is the group of chief dynamic components and has number of medicinal uses such as anti-inflammatory, anti-HIV, antitumour, antiviral, anticancer, antifungal and antiparasitic. Different analytical methods have been developed in recent year for the quality control analysis of curcuminoids in Curcuma longa extract including HPLC, HPTLC and UV-Visible Spectrophotometry. While the primary component curcumin from curcuminoids is still lacking for its analytical method development along with validation. Therefore, in the present study, a simple UV visible and HPLC method was developed and validated according to international conference harmonization (ICH) guidelines for the quantitative estimation of curcumin in Curcuma longa extract.
7.	Curcumin and	UV	• Detection Wavelength: 422 nm and 266 nm.

	Ascorbic acid	Spectroscopy	• Linearity: of 2 to 10 µg/mL for both curcumin and ascorbic acid with a correlation coefficient of 0.9992 and 0.999
8.	Curcumin and quercetin	UV Spectrophotometry and HPLC	• Methanol: acetonitrile: phosphate buffer (42.5: 42.5: 15 % v/v/v) • Detection wavelength: 265 nm • Recovery:98.50-99.40%
9.	Curcumin and acyclovir	HPLC	• Column: Fenomenex C18 Mobile phase: ternary mixture of acetonitrile, 0.1% phosphoric acid, and methanol (50:40:10) • Linearity range: 0.5-30 µg/mL (Acyclovir), 0.5-20 µg/mL (Curcumin)
10	Ascorbic acid	UV	A UV method for the analysis of ascorbic acid with methanol as solvent to prepare a sample has been developed and applied. The effect of copper (II) concentrations on the oxidation of ascorbic acid in aqueous solution has been studied in detail, and the regularities of ascorbic acid oxidation in methanol, USP phosphate buffer (pH 2.50) and de-ionized water have been found. Upon experiments ascorbic acid has been found to dissolve in methanol, and its solubility in it has been measured to be 81.0mg/ml at room temperature (22 °C). The ascorbic acid bulk material from a manufacturer has been assayed to be 89.34% with this method, in good agreement with the assay value (89.58%) from the titration method.
11	Ascorbic acid	HPLC	• Column: Superspher RP-18 (250 mm x 4.6 mm, 10ìm particle size) at 20 °C. • Mobile phase: Acetic acid (500 ml) to 1.5g of 1-hexanesulfonic acid sodium salt and mixing well (pH 2.6). • Flow rate: 0.7 mL min-1. • Detection Wavelength: 280 nm • LOQ: 1.95 µg/mL • Recovery: 99.58% to 101.93%
12	Ascorbic acid and gallic acid	HPLC	• Column: C18 • Mobile phase: methanol and 0.1% (v/v) acetic acid in HPLC-grade water as mobile phase • Flow rate :0.9 mL min^{-1} • UV detection: 278 nm • Linearity range: 30-90 µg/mL (Ascorbic acid), 5-15 µg/mL (Gallic acid)
13	Aspirin, salicylic acid and caffeine	HPLC	• Column: Hypersil C18 (5 µm, 15 cm × 4.6 mm) Mobile Phase: water–methanol–acetic acid • Detection wavelength: 275 nm • Overall Recovery is 100.2%, 100.7%, and 99.2% for aspirin, caffeine, and salicylic acid
14	Aspirin and folic acid	HPLC	• Column: C18 using a photodiode array (PDA) detector • Wavelengths :233 nm (salicylic acid) and 277 nm (folic acid). • Mobile phase: Acetonitrile − 0.1% trifluoroacetic acid mixture programmed for a 30 min gradient elution analysis
15	Salicylic acid,	HPLC	• Column: A 150 mm × 4.6 mm I.D. YMC J'sphere ODS-

	betamethasone dipropionate		H80 • Temperature: 35 °C, Detection Wavelength: 240 nm • Mobile phase: 0.05% (v/v) methanesulfonic acid solution and acetonitrile
16	Ascorbic acid in health drinks	RP-HPLC	• Column: C18 (250x4.6mm, 0.5µ in particle size) ambient temperature. • Mobile Phase: Water with acetic acid: methanol 95:5% (v/v). • Detection Wavelength: 245 nm • Injection volume :20µl
17	Ascorbic acid and salicylamide	RP-HPLC	• Column: CLC Shim-pack C8 (250 x 4.6 mm, 5 µm particle size) • Mobile phase: methanol: 0.03 M phosphate buffer mixture (55: 45, v/v) pH 4.0 • Flow rate: 1 mL/min • Detection Wavelength: 255 nm
18	Ascorbic acid and folic acid vitamins	RP-HPLC	• Isosbestic point: wavelength 280nm • Retention time: 2.334 min (ascorbic acid) and • 3.892 min (folic acid)
19	Ascorbic acid, phenylephrine, paracetamol and caffeine	HPLC	• Colmun: Monolithic C18 (100 × 4.6 mm), • Mobile phase: acetonitrile and phosphate buffer (pH 6.50) (10: 90, v/v) • Flow rate: 1.0 mL min^{-1} and temperature: 25 °C, • Detection was observed at two wavelengths 210 nm (phenylephrine, paracetamol and salicylic acid) and 235 nm (ascorbic acid and caffeine)
20	Iron and ascorbic acid	HPLC	• Colorimetric method for estimation of total iron: The wave length used was 510 nm with diluent-1 as 0.5 M sulfuric acid and 1.2 M sodium acetate 5 mL. The diluent-2 was Milli Q-water. • HPLC: • Column: Inertsil octadecylsilyl (250 mm ×4.6 mm; 5 µm) 20 mM • Mobile Phase: potassium dihydrogen phosphate (pH - 2.5) and methanol (97:03). • An isocratic program was followed using 0.3 M hydrochloric acid with 5% ortho phosphoric acid as diluents with 1 ml/min flow rate.
21	Salicylic acid	Spectrophoto metric	• Detection Wavelength: 301.2 nm. • Linearity range: 5-60 µg/ml. • LOD: 1.52 µg/ml • LOQ: 4.60 µg/ml
22	Salicylamide, salicylic acid and deferasirox	HPLC	• Column: Waters symmetry C18 (250 cm × 4.6 mm, 5 µm) • Mobile phase: buffer and acetonitrile 40:60 (v/v) with apparent pH adjusted to 3.2 • Detection Wavelength: 245 nm