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A SYSTEMATIC REVIEW OF DIFFERENT ANALYTICAL METHODS FOR MAJOR PHYTOCONSTITUENTS OF TURMERIC AND BLACK PEPPER

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ABSTRACT: Curcumin is a phytochemical isolated from Curcuma longa (Zingiberaceae) and has been used by ancient people for a long time to treat various ailments. Piperine is the main bioactive constituent that has been isolated from *Piper nigrum L*. Piperine increases the bioavailability of curcumin. Several analytical methods have been developed for the detection of curcumin and Piperine like high-Performance Liquid Chromatography (HPLC), high-performance thin layer chromatography (HPTLC), ultraviolet (UV) spectroscopic methods, Ultra-performance liquid chromatography (UPLC), and hyphenated methods. Numerous analytical techniques have been developed to quantify curcumin and piperine; most focus on accomplishing this separately. Only a few techniques have been created to determine curcumin and piperine in the combined dosage form simultaneously. The main purpose of this review is to describe a brief overview of the method development for Curcumin and Piperine in pharmaceutical dosage form, biological fluids, and Herbal formulations.

INTRODUCTION: Our ancestors have been treating various ailments with medicinal plants since ancient times, and it is still practiced in many places. To date, 35000 to 700000 species are being used for curing various diseases, and their demand is increasing globally. The World Health Organization (WHO) backs efforts in research and training to ensure that traditional medicines are used safely and effectively [1]. All companies have a standard parameter to follow to maintain the standard product quality; that's why validation of the product is done in every batch possible.

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Analytical methods development and validation play important roles in discovering, developing, and manufacturing pharmaceuticals. Quality control laboratories use the official test methods that result from these processes to ensure the identity, purity, potency, and performance of drug products [2]. Natural plant products can treat a wide range of infections and ailments. One such example is the Zingiberaceae family's turmeric, which ancient peoples have used for a long time.

Turmeric (*Curcuma longa Linn*) is a plant that is grown all over the world in tropical and subtropical environments. India, Southeast Asia, and Indonesia are their native habitats [3]. Of all these countries, Curcumin (CUR) is mostly cultivated in India (93.7%of the total world production). *Curcuma longa* contains a group of economically important taxa as medicine, food, coloring, and ornamental materials [4]. As India has a rich diversity in its

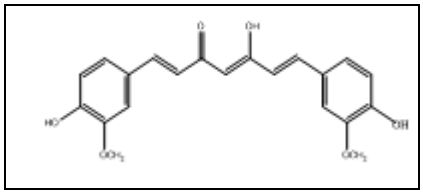
geographical areas, it was found that the content of the curcuminoids found in the turmeric of different places varied. Variability in the concentration of curcuminoids could be due to changes in different environmental conditions of the Indian subcontinent. The results showed that maximum and minimum amounts of curcuminoids were present in the Erode (south province) and Surat (west province) samples, respectively [5]. *C. longa* extract has three important compounds, curcumin (Cur), demethoxycurcumin, and bisdemethoxycurcumin, present in approximately 70–77%, 18–20%, and 5–10 %. Curcuminoids are a mixture of curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) [6]. CUR is Chemically described as (1E, 6E)-1.7-bis (4 hydroxy – 3 methoxyphenyl) – 1,6 Heptadiene-3,5-dione. It is naturally hydrophobic and frequently soluble in dimethylsulfoxide, acetone, ethanol, and oils. It has an absorption maximum of around 420 nm [7].

Years of research when putting together it was found that it contains Antibacterial Activity [8] antipathogenic and antiparasitic activities [9] antibacterial, antiviral, and antifungal activity [10] antioxidant activity [11] various central nervous system (CNS)-related diseases including Parkinson's disease, Huntington's disease, Alzheimer's disease, Multiple sclerosis, epilepsy, and Amyotrophic Lateral Sclerosis 12anti-cancer properties [13].

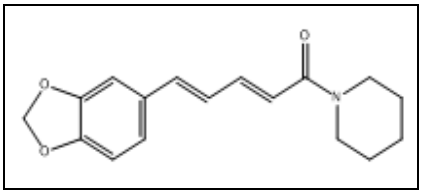
Black pepper (*Piper nigrum* L.), often known as "black gold," "pepper," and "king of spices," is one of the most extensively used spices. Piperine (PIP; an alkaloid) is the main bioactive constituent that is considered the pungent principle of the plant; that is, it imparts a pungent flavor. *P. nigrum* is a flowering vine that belongs to the Piperaceae family and has been reported to contain a variety of naturally occurring phytochemicals, with PIP (an alkaloid) being the main bioactive constituent that is considered the pungent principle of the plant [14]. PIP is a yellow crystalline chemical with the IUPAC name 1-(5-[1,3-benzodioxol-5-yl]-1-oxo-2,4-pentadienyl) piperidine [15]. PIP is a plant-derived alkaloid well-known for its broad range of biological actions. PIP is well known for its antidepressant, antipyretic, analgesic, anti-inflammatory, antioxidant, hepatoprotective, and anti-diabetic properties demonstrated in clinical and pharmacological research [16]. PIP (trans–trans) is one of four geometric isomers that include two double bonds, including iso-piperine (cis-trans), chavicine (cis-cis), and iso-chavicine (trans–cis). PIP gives black pepper its pungency, even though its counterparts (piperanine, piperettine, piperylin A, piperolein B, and pipericine) have also been discovered [17]. The Western Ghats of the South India Peninsula is the principal focus of black pepper cultivation geographically. Black pepper cultivation has spread to other nations in South and Southeast Asia since then. Black pepper is thought to have originated in India, in the Western Ghats' submontane regions. It is mostly grown in tropical countries such as India, Malaysia, Indonesia, and Brazil, as well as to a smaller extent in Sri Lanka and the West Indies. The total export from the various producing countries averages around 138,000 t. Some major producing countries include India, Indonesia, Malaysia, Sri Lanka, Vietnam, and Brazil.

From Guinea to Uganda, black pepper is widely used [18]. Both black and white pepper are used as flavoring agents in homes and can be purchased in various forms, including crude, whole, or crushed pepper fruits, oil, and oleoresin. Regarding FDA regulatory status, all kinds of pepper are usually considered safe, with the greatest average use levels observed in nut products (0.42 percent white pepper) and baked goods (0.2% oleoresins) [19].

CUR and PIP are two dietary polyphenols investigated for their anti-cancer effects, particularly colorectal cancer (CRC). Curcumin is more effective when PIP is added to it [20]. CUR and PIP have poor light stability, so all solutions were prepared in amber glassware [21]. Although several analytical methods for quantifying CUR and PIP have been developed, most of these methods are focused on quantifying CUR and PIP separately. Only a few methods have been developed to determine CUR and PIP simultaneously in the combined dosage form. This paper focuses on various analytical methods developed for the determination of CUR and PIP individually and in combined dosage form.



Curcumin



Piperine

TABLE 1: THE DIFFERENT SPECTROPHOTOMETRIC METHODS WHICH HAVE BEEN USED FOR THE ANALYSIS OF CUR ARE SUMMARIZED IN THE TABLE BELOW

Sl. no.	Study Objective	Method used	Parameter	Results	Refe.
1	Simultaneously estimate CUR and PIP in their combined nanoparticulate system (2021).	UV Spectrophotometric Method	Solvent used: methanol used as a solvent. standard calibration curve of CUR and PIP: CUR and PIP calibration curve was plotted at a concentration range of 1–30 µg/ml. The methods mentioned have been validated using International Conference on Harmonization (ICH) parameters.	Determination of λmax and Iso-absorptive Point: CUR=423 nm PIP =342 nm iso-absorptive point = 368.5 nm. calibration curves CUR and PIP show linearity of 1–7 µg/ml Accuracy CUR=100.034% and 101.328% and PIP=100.665% and 102.247% for PIP. LOD CUR=0.092 µg/ml. PIP= 0.102 µg/ml. LOQ: CUR=0.280 µg/ml PIP=0.311 µg/ml. LOD (iso-absorptive point) CUR=0.0683 times PIP= 0.1024 µg/ml, LOQ (iso-absorptive point) CUR=0.207 µg/ml PIP=0.310 µg/ml. Assay of CUR+PIP in NLCs formulation was found to be 96.80% and 97.55%.	[22]
2	Estimation of CUR in simulated nasal fluid (2020).	UV Spectrophotometric Method	Solvent system: simulated nasal fluid with two surfactants (tween 80 and sodium lauryl sulphate).	Selection of Wavelength: 421 nm. Linearity: range 5– 60 µg/ml (0.998). Accuracy range found 99.51 –100.223 %. LOD=0.3657 LOQ =1.109	[23]
3	Characterization of CUR loaded nanostructured lipid nanocarriers in simulated conditions (2019).	UV Spectrophotometric method	Solvent used: methanol and 6.4 pH phosphate buffer with 1.5% polysorbate UV spectroscopy scanning (800-200nm) was performed.	Maximum absorption 425 nm for both solvent systems. Linearity: CUR in both the media was 1.0–11.0 µg/mL with a correlation coefficient (R^2) greater than 0.998. LOD=0.19 µg/mL. LOQ = 0.57 µg/mL. Accuracy range of 99.01 - 100.1% and the RSDs less than 0.5%	[24]
4	Development and validation of an analytical method for CUR in polyherbal formulation (2017).	UV Spectrophotometric method	Determine of λmax of CUR: 10µg/ml solution they prepare and scan at the range of 400-600 nm. Ethyl acetate is used as a black. Calibration curve: Standard solutions in ethyl acetate in the 1-5 µg/mL range were measured at 418 nm.	λmax: CUR 418 nm. Linearity range: 1-5 µg/ml. Coefficient of correlation- 0.999. Recovery values for the standard addition method followed for the CUR analysis ranged from 98.4100 to 101.000%	[25]

TABLE 2: THE DIFFERENT CHROMATOGRAPHIC METHODS WHICH HAVE BEEN USED FOR THE ANALYSIS OF CUR ARE SUMMARIZED IN THE TABLE BELOW

Sl. no.	Study Objective	Method used	Parameter	Results	Ref.
1	Simultaneous Quantification of CUR and D-Panthenol (2022).	RP-HPLC-UV method	Preparation of Stock, Calibration Standards: CUR and DPA stock solutions were separately prepared in methanol and Phosphate buffer solution. Linearity check 0.19 to 12.5 µg mL^{-1} for CUR and from 0.19 to 25 µg mL^{-1} for DPA. Chromatographic Conditions. Column: C18 column. Mobile phase: 0.001% v/v of phosphoric acid in water (mobile phase A) Acetonitrile (ACN) (mobile phase B). Injection volume = 20 µL Analysis time=15min	Linearity: concentrations range from 0.39–12.5 µg mL^{-1} (R^2 = 0.9999) for CUR and 0.39–25 µg mL^{-1} for DPA (R^2 = 1)	26
2	Simultaneously quantify CUR and PIP content in solid dispersion-based microparticle formulation containing *Curcuma longa* and Piper nigrum extracts (2021).	RP-HPLC method	Finding the detection wavelength: methanolic solutions of CUR 15 µg/mL and PIP (5 µg/mL) were scanned at the range of 315-450 nm using a UV-Vis spectrophotometer. Chromatographic Condition C18 column injection volume was 20 µL. Mobile phase: Acetonitrile methanol-water of 65:5:35 %, Flow rate = 1 mL/min. wavelength = 353 nm for detection.	Resolution (Rs) CUR=1.68. PIP=3.57. Theoretical plate number (N) > 8000. Tailing factor (T) < 1.5. Calibration curve: CUR=2.5- 30 µg/ mL. PIP =1.25-15 µg/mL. The limit of detection (LOD) was 0.27 and 0.42 µg/mL, for PIP and CUR. Developed method recovery 91.14% and 99.14% for PIP and CUR	27
3	Determination of a mycophenolic acid-curcumin (MPA-CUR) conjugate in buffer solutions (2021).	RP-UPLC method	Chromatographic Condition. Column = C18 column. Solvent: water and acetonitrile, each containing 0.1% formic acid, Flow rate of 0.6 mL/min,Column temperature at 33 ◦C. Compounds detected simultaneously at the maximum wavelengths of mycophenolic acid (MPA), 254 nm, and CUR, or MPA-CUR, at 420 nm	Calibration curves: Linearity ranges from 0.10 to 25 µg/mL ($r^2 \geq 0.995$). LOD= 0.04 µg/mL. LOQ = 0.10 µg/mL. The accuracy and precision of the developed method were 98.4–101.6%, with %CV < 2.53%.	28
4	Simultaneous determination of CUR and Quercetin in the nanoemulsions (2020).	RP-HPLC–UV/Vis method	Chromatographic Conditions The column used: C_{18} column. Mobile phase: Aqueous phosphoric acid 1% w/v adjusted at pH 2.6 (Eluent A): Acetonitrile (Eluent B) Flow rate 1.0 mL/min. Detection wavelength. 400 nm. The solvent used to prepare standard solution. Methanol and 1% phosphoric acid	Linearity range. CUR =0.25–12.5 µg/mL (R^2 > 0.9997) Quercetin =0.25–12.5 µg/mL (R^2 > 0.9997) precise (RSD below 3% LOD: CUR=0.005 µg/mL QU=0.14 µg/mL LOQ: CUR =0.017 µg/mL QU=0.48 µg/mL. CUR and QU encapsulation efficiencies > 99%	29
5	Determination of hydrazinecurcumin in rat plasma and organs (2020)	RP-HPLC method	Chromatographic conditions Column= Inertsil® ODS -3V Mobile phase: methanol-acetonitrile- water (36:27:37 v/v). Flow rate 1.0 ml/min injection volume= 50 µl wavelength = 332 nm and 380 nm for hydrazinocurcumin and p-nitro aniline. The solvent used for Prepare stock solution: methanol (HPLC grade).	Linearity: Hydrazinocurcumin range 0.05-5 µg/ml (r^2>0.999). inter-day accuracy (98.04% to 105.94%). precision (0.89 to 10.24). recoveries of hydrazinocurcumin . rat plasma= 96%–101.75% various organs= 92.25% to 99.0%,	30
6	CUR evaluation in plasma of supplemented athletes (2020)	RP-HPLC-FLD method	Chromatographic condition: Column Used: C18 Luna column coupled with a Security Guard Gemini C18 column mobile phase: acetonitrile: acetic acid pH 3.2 (45:55 to 60:40). Flow rate= 1 mL min^{-1} Injection volume= 10 µL	The method was linear between 44 and 261 ng mL^{-1}showing intra-day (2.05.6%) and inter-day (4.0–5.1%) precision. accuracy and selectiveness (CUR t_R = 8.7 min and internal standard t_R = 13.9 min with relative recovery of 83.2%)	31

7	Simultaneous Determination of Irinotecan Hydrochloride and CUR in Co-delivered Polymeric Nanoparticles (2020).	RP-HPLC method	Chromatographic condition: Acetonitrile and ultrapure water containing sodium dodecyl sulfate (0.08 mol/L), disodium phosphate (Na2HPO4, 0.002 mol/L), and acetic acid (4%, v/v) (50:50). Flow rate: 1.0 mL/min, Column Temperature: 40ºC. The analysis was carried out at 256 and 424 nm to assess irinotecan hydrochloride and CUR	Retention times Irinotecan hydrochloride=3.317 min. CUR =5.560 min Recovery=100 ± 2%. Precise (RSD ≤ 1%) Robust and linear ($R^2 \geq$ 0.9996) in the range from 2.05 to 1050 µg/mL.	32
8	Determination of CUR diethyl disuccinate, an ester prodrug of CUR, in raw materials (2020).	RP-UPLC method	Chromatographic separation Column used: Acquity UPLC® BEH C18 Detection wavelength 400 nm. Mobile phase: 2%v/v acetic acid in water and acetonitrile Flow rate = 0.3 mL/min	Linearity range: Over 8–12 µg/mL with the coefficient of determination >0.995. %Recovery ranged from 98.3 – 100.8%. The precision of the method expressed as %CV was found to be <1%	33
9	Quantify atorvastatin and CUR after their intranasal co-delivery to mice (2019).	RP-HPLC method	Chromatographic Condition: Column used=C18 column Mobile phase: Acetonitrile-methanol-2 % (v/v) acetic acid (37.5:2.5:60, v/v/v) Flow rate =1.0 mL/min. Detection wavelength analytes and internal standards were carried out at 247 nm, 425 nm, and 250 nm, respectively.	Limits of quantification CUR:10 to 500 ng/mL. Atorvastatin :100 to 600 ng/mL. Linear over a wide concentration range ($r^2 \geq$ 0.9971). Accuracy (bias ± 12.29%). Precision (coefficient of variation ≤ 13.15%). Analytes were recovered at a percentage higher than 81.10% and demonstrated to be stable on several experimental conditions in all biological matrices	34
10	Simultaneous Determination of CUR and Resveratrol in Lipidic Nanoemulsion Formulation and Rat Plasma (2019).	RP-HPLC method	Chromatographic Condition: The column used: C18 column Mobile Phase: using acetonitrile (ACN)–water (pH adjusted to 4.6 by 1% orthophosphoric acid) in the ratio of 55:45 (v/v). Flow rate = 0.8 mL/min Detection wavelength: CUR= 425nm resveratrol=304 nm	Extraction efficiency CUR=96.10–101.00% (RSD 2.49). Resveratrol=95.00–99.87% (RSD 2.59). The assay was linear from 0.05 to 4.00 µg/mL (correlation coefficient of 0.9989 and 0.9981, respectively). Average interday and intraday precision: CUR RSD% (0.45, 2.04) Resveratrol RSD% (2.25, 1.71)] in spiked rat plasma. LOD and LOQ: CUR: (0.0085 µg/mL, 0.025 µg/mL). PIP: (0.02, 0.06 µg/mL), respectively	35
11	Quantitative assessment of turmeric extracts from different geographical locations (2019).	RP-UPLC-DAD method	Chromatographic Condition Column: Acclaim RSLC Polar Advantage II column . Detection wavelength at 425 nm. column temperature 45◦C. Mobile phase acetonitrile and water, Flow rate 1 mL/min	calibration curve: Each curcuminoid showed good linearity (correlation coefficient > 0.999). The relative standard deviation of intra-day, inter-day precision, and repeatability 0.73%, 2.47% and 2.47%, respectively. Accuracy ranged from 98.54%-103.91% with RSD values of less than 2.79%.	36
12	Simultaneous quantification of CUR and its metabolites in plasma and lung tissue (2018).	RP-HPLC-UV method	Chromatographic parameters Atlantis® dC18 guard column mobile phases Ammonium acetate, pH 4.5 (mobile phase A) Acetonitrile (mobile phase B) Injection volume:50 µL, Flow rate = 1 mL/min, Autosampler temperature at 4°C	Calibration curves spanned seven concentrations (0.05, 0.1, 0.2, 0.5, 1, 2, 5 µg/mL), and were linear for all analytes in both the matrices. The average correlation coefficient (r^2) exceeded 0.998 (n = 3), Accuracy values for the entire range of calibration standards of ±15%, and thus within acceptable limits. The retention times CUR glucuronide=15.8 mins. CUR sulfate=19.3 mins. CUR= 23.7 mins. LLOQ of the assay was determined by assessing the lowest	37

				concentration in the standard curve that could be quantified with 80-120% accuracy and precision	
13	Simultaneous Assay of Curcuminoids and Doxorubicin from Long Circulating Liposome (2018).	RP-HPLC method	Chromatographic system Column used: Zorbax SB C18 column Mobile phase: formic acid 0.2%-ACN system (pH = 2.3) Detection wavelengths 490/560 nm for DOX and 420/470 nm for curcuminoids, respectively	Linearity range:2-20 µg/ml for all investigated compounds. The intra-and inter-day precision were less than 2%, Accuracies between 97-104% of the true values.	38
14	Simultaneous Quantification of Three Curcuminoids and Three Volatile Components of *Curcuma longa* (2018).	RP-HPLC method	HPLC conditions Column: Zorbax SB-C18 column Mobile phase Acetonitrile and 0.4% (v/v) aqueous acetic acid Eluting rate of 1.0 mL/min curcuminoids and volatile components were detected at 430 nm and 240 nm	The calibration curves analytes showed good linearity (R^2 > 0.9999) LOD and LOQ of the six analytes were 0.20–0.91 µg/mL and 0.67–3.02 µg/mL, respectively.	39
15	Determination and stability study of CUR in pharmaceutical samples (2017) .	RP-HPLC method	Chromatographic Condition: Mobile phase: acetonitrile: ammonium acetate (45:55, v/v, pH 3.5). column: C18 column (150 mm×4.6 mm×5 µm particle size). Flow rate: 1 ml/min in ambient condition retention time = 17 min. volume of injection 20 µl. Detection wavelength: 425 nm.	linearity range: 2-100 µg/ml. LOD and LOQ were 0.25 and 0.5 µg/ml, The percentage of recovery: 98.9 to 100.5 with a relative standard deviation (RSD) < 0.638%	40

TABLE 3: THE DIFFERENT HPTLC METHODS WHICH HAVE BEEN USED FOR THE ANALYSIS OF CUR ARE SUMMARIZED IN THE TABLE BELOW

Sl. no.	Study Objective	Method used	Parameter	Results	Ref.
1	CUR in the methanolic fraction of *Curcuma longa* L. rhizomes were analyzed (2021).	HPTLC method	Standard preparation: 0.1 mg mL−1 of CUR in the methanol. Instrumentation and chromatographic conditions: HPTLC plates: 20 × 10 cm, 0.2 mm thickness pre-coated with the silica gel 60 F254 (E-Merck). Scanning speed: 10 mm s−1. Sample volume: 10 µL. Solvent system: chloroform: methanol (97:3V/V). Detection wavelength: 420 nm. Calibration curve of CUR: 100–600 ng per band.	Absorption spectrum: CUR 420 nm. Linearity: 100–600 ng per spot & r^2 value 0.9998. Rf value: 0.37±0.04. LOD & LOQ LOD (ng/spot) =35. LOQ (ng/spot) = 100	41
2	Validation and quantification of major biomarkers in 'Mahasudarshan Churna'- an ayurvedic polyherbal formulation (2020).	HPTLC method	Mahasudarshan Churna(MC) composition: oleanolic acid (OA), ursolic acid (UA), mangiferin (M), gallic acid (GA), quercetin (Q) and curcumin (C). The mobile phase, hexane: ethyl acetate: acetone (16.4: 3.6: 0.2, v/v) was used for the separation of OA and UA; ethyl acetate: glacial acetic acid: formic acid: water (20: 2.2: 2.2: 5.2 v/v) for the development of M; and toluene: ethyl acetate: formic acid (13.5: 9: 0.6 v/v) for the separation of GA, Q, and C in crude sample extracts. Visualization and scanning were performed at λ = 530 nm for OA and UA, at λ = 254 nm for M, and at λ = 366 nm for GA, Q and C. HPLC-PDA analysis was used to confirm the HPTLC results.	Major bio-active compounds in MC formulations: oleanolic acid (1.54–1.78%), mangiferin (1.38–1.52%) and gallic acid (1.01–1.15%); followed by ursolic acid (0.79–0.98%), CUR (0.45–0.67%) and quercetin (0.22– 0.34%)	42

TABLE 4: THE DIFFERENT HYPHENATED TECHNIQUES WHICH HAVE BEEN USED FOR THE ANALYSIS OF CUR ARE SUMMARIZED IN THE TABLE BELOW

Sl. no.	Study Objective	Method used	Parameter	Results	Ref.
1	Simultaneous quantification of	LC-ESI-MS/MS	Chromatographic Condition Column: XBridge BEH C18	Linearity: curcumin-O-sulfate =0.5-1000 ng/mL and curcumin-	43

	free curcuminoids and their metabolites in equine plasma (2018).	method	column. Mobile phase: acetonitrile containing 0.1% (v/v) formic acid] (Mobile Phase A) water containing 0.1% (v/v) formic acid (Mobile Phase B). Flow rate: 0.2 mL/min injection volume 8 µL. The column temperature was maintained at 40 °C.	O-glucuronide = 1-1000 ng/mL with 85-115% accuracy and <15% precision in equine plasma	
2	Simultaneous determination of epigallocatechin-3-gallate, silibinin, and CUR in plasma and different tissues after oral dosing of Protandim in rats (2018).	LC-MS/MS method	Chromatographic Condition Column: Hypurity C18 column) Mobile phase water and acetonitrile. Mass spectrometric detection was performed in the multiple reaction monitoring mode (MRM) following the transitions: m/z 457.3/169.3, m/z 481.3/125.0, m/z 367.3/149.3 and m/z 609.4 /300.2 for EGCG, SIL, CUR, and RU (rutin), respectively.	Linearity: all analytes in the range from 2 to 1000 ng/mL. For all matrices, the values of inter-day and intra-day precisions and accuracies were less than 10.3 % of the nominal concentration. The matrix effect, extraction recovery, dilution integrity, and stability values were all within acceptable levels.	[44]
3	simultaneous determination of CUR, curcumin glucuronide, and curcumin sulfate in phase II clinical trial (2018).	LC-MS/MS method	plasma samples: prepared by methanol. Condition: Column: Waters XTerra® MS C18 column Mobile Phase: methanol and 10.0 mM ammonium formate (pH 3.0) flow rate 0.250 Ml min^{-1}	calibration range: was 2.50-500 ng mL^{-1} for CUR, COG, and COS.	[45]

TABLE 5: FLUOROMETRIC METHOD WHICH HAVE BEEN USED FOR THE ANALYSIS OF CUR ARE SUMMARIZED IN THE TABLE BELOW

Sl. no.	Study Objective	Method used	Parameter	Results	Ref.
1	CUR Determination in Nanoliposomes and Plasma: Development and Validation (2020).	Spectrofluoro metric method	Standard Solution: dissolved in DMSO and was vortexed until complete dissolution of CUR. The resultant solution was diluted using methanol. Linearity and Range: six points were selected ranging from 0.05 to 0.5 µg/mL. Excitation and emission wavelengths were set at 423 nm and 527 nm, respectively	The calibration curve was linear for CUR concentrations of 0.05 to 0.5 µg /mL with a correlation coefficient of 0.9996. The limit of detection (LOD) and limit of quantification (LOQ) were 0.03 and 0.10 µg/mL, respectively	[46]

TABLE 6: THE DIFFERENT SPECTROPHOTOMETRIC METHODS WHICH HAVE BEEN USED FOR THE ANALYSIS PIP ARE SUMMARIZED IN THE TABLE BELOW

Sl. no.	Study objective	Method used	Parameters	Results	Ref.
1	Determination of the Dissolution Behavior of Solid Dispersions Containing CUR and PIP (2018).	UV Spectrophotome tric Method	stock solution: stock solutions were produced in methanol and were diluted with sodium lauryl sulphate in phosphate buffer; pH 6.0). UV-VIS spectrophotometer, solutions of CUR (2g/mL) and PIP (1g/mL) were overlay scanned at 430 and 335.5 nm for CUR and PIP, respectively	Linearity: Range 0.1 to 5g/mL. CUR and PIP calibration curves revealed linearity with R^2 = 0.9980 and 0.9982, respectively. LOD of both substances was 0.23 g/mL, LOQ: CUR=0.72 g/mL. PIP=0.69 g/mL.	[47]
2	Method development for estimation of PIP in polyherbal lozenges (2016).	UV Spectrophotome tric Method	stock solution: PIP was dissolved in methanol. Water is taken as blank. They selected three lozenges of different brands and named them S1, S2 & S3 which contained a different amounts of piperine.	Concentration range = 2-20 µg/ml. Calibration equation was found to be Abs =0.07733Concentration. Correlation Coefficient =0.99569. %Recovery of	[48]

			Absorbance measured = 342nm. Mobile phase used : chloroform and methanol(95:5)	S1,S2,S3 was found to be 98.62%, 99.32% and 98.35 % respectively	
3	Simultaneous estimation of isoniazid, rifampicin, and PIP in pharmaceutical dosage form (2014).	UV-spectrophotometric and RP-HPLC method	Solvent used: 1st diluent used was methanol and distilled water. The wavelength selected: 262 nm, 33,8 nm and 477 nm for INH,, PIPE and RIFA respectively. For RP – HPLC method Chromatographic Condition: Column used: LC18 100 Aº column (Mobile phase: acetonitrile (40:60, % v/v). Flow rate: 0.9 mL/min. Wavelength detection =282 nm.	Linearity range for absorption correct method:12-34.5 µg/mL (INH), 8-23 µg/mL (RIFA and 0.4-1.15 µg/mL (PIPE) respectively. For RP – HPLC method 30- 330 µg/mL (INH), 20-220 µg/mL (RIFA) and 1-11 µg/mL (PIPE) R^2 = >0.995. Accuracy = 98%-102%	[49]
4	Simultaneous Estimation of Rifampicin and PIP in their Combined Capsule Dosage (2012).	UV Spectrophotometric method	Preparation of standard: methanol is used as a solvent Wavelength selected e RIFA and PIP showed an isoabsorptive point at 387 nm. Another wavelength 337nm was taken and absorption was calculated using calibration curve.	Linearity curve: Rifampicin:5-40 µg/ml PIP:2-20 µg/ml drugs). Regression equation: Rifampicin: y = 0.003x + 0.010. PIP: y = 0.009x + 0.010 LOD (µg/ml) & LOQ (µg /ml): 0.80 & 0.32 respectively	[50]
5	Quantitative analysis of PIP in ayurvedic formulation (2010).	UV Spectrophotometry method	A calibration curve was created using a standard solution of PIP, and the content of PIP in Sitopaladi churna was determined using this curve. The method's precision and accuracy were verified	Absorption maxima =342.5nm. Beer's law limit= 10-50ug/ml. Regression equation = (y=bx+a) 0.013x+0 Correlation coefficients(r2) 0.9961 Precision (n=6, % RSD) =0.978 Accuracy (%) = 99.03	[51]
6	Development for estimation of PIP in Chitrakadi Vati (2010).	UV-spectrophotometric method	To estimate the percent w/w of PIP, three commercial Chitrakadi Vati formulations comprising black pepper (Piper nigrum) and pippali (Piper longum) from different manufacturers (CV-1, CV-2, and CV-3) were used in this investigation. Preparation of stock solution: PIP stock solution was made by dissolving PIP in methanol. PIP standard solutions in the 2- 20 g/ml concentration range were produced in volumetric flask using methanol as the solvent. UV absorbance: 342nm	Linearity range: 2-20 µg/ml Regression value = 0.9956 Calibration equation Y = 0.07733*X -0.04974 CV-1, CV-2, and CV-3 marketed preparations was determined to be 0.1032 percent, 0.0852 percent, and 0.0898 percent w/w, respectively. Recovery % CV-1= 98.51%, CV-2=99.12% CV-3=98.92%	[52]

TABLE 7: THE DIFFERENT CHROMATOGRAPHIC METHODS WHICH HAVE BEEN USED FOR THE ANALYSIS OF PIP ARE SUMMARIZED IN THE TABLE BELOW

Sl. no.	Study objective	Method used	Parameters	Results	Ref.
1	Simultaneous estimation of aloe-emodin and PIP in ayurvedic formulation (2019).	RP-HPLC method	Chromatographic Conditions Column used: Prontosil C_{18} Mobile phase: 0.05%orthophosphoric acid and acetonitrile in a 50:50 ratio was used. Flow rate = 1 ml/min, Column temperature maintained at 28°C, and ultraviolet (UV) detection wavelength at 225 nm.	Retention time of aloe-emodin and PIP = 9.38±0.2 min and 13.45±0.2 min, respectively. Linearity of aloe-emodin and PIP range =1–20 µg/ml. Correlation coefficient for aloe-emodin and PIP =0.998 and 0.997, respectively. Recovery values = (98–102%) indicate a satisfactory accuracy. The percentage relative standard deviation for precision was found to be <2%.	[53]

2	Analytical method by liquid chromatography to assay PIP associated in nanoemulsions (2018).	RP-HPLC with UV detection method	Chromatographic Conditions Mobile phase: Methanol:water was used as a solvent. Isocratic flow = 1.0 mL min^{-1} UV detection = 343 nm & the equipment was operated at room temperature. Preparation of stock solution: Methanol used as a solvent	Linearity range: 5 to 50 µg mL^{-1} Correlation coefficient: (r^2 = 0.9999; y = 258166x − 103392) LOQ & LOD 1.26 µg mL^{-1} and 0.41 µg mL^{-1}, respectively For repeatability and intermediate precision, the relative standard deviation (RSD) values found were 0.38 percent and 1.11 percent, respectively. Recovery test was used to assess the accuracy, and the average percentage found was 101.35±0.80 percent	54
3	Bioanalytical method development, validation and its application in pharmacokinetic studies of verapamil in the presence of PIP in rats (2018).	RP-HPLC method	Chromatographic Conditions Column used: RP C_{18} column, Mobile phase: acetonitrile: ammonium acetate buffer (pH 5.6 adjusted with acetic acid)	λmax: CUR 418 nm. Linearity range:1-16 µg/ml Coefficient of correlation- 0.998. UV detection: 288 nm. Limit of detection & limit of quantitation = 0.32 µg/mL and 0.96µg/mL, respectively. The Cmax and AUC of verapamil were observed to increase by 154% and 135%, respectively.	55
4	Development and validation of stability indicating simultaneous estimation of CUR and PIP in bulk mixture (2016).	RP-HPLC method	Chromatographic Conditions Column used: Synchronis C-18 column. Mobile phase: Acetonitrile: Water (0.1% Acetic acid, pH 3.2 in 60:40 v/v). Preparation of stock solution: Methanol used as a solvent Method was validated in terms of linearity, specificity, precision, accuracy, limit of detection and limit of quantification	Linearity range : CUR=25-125µg/ml PIP=0.251.25µg/ml. Regression coefficient: CUR: 0.9979 PIP:0.999. Detection wavelength =343nm. Flow rate = 1ml/min. Limit of Detection (LOD). CUR =8.4167 PIP= 0.18628 Limit of Quantitaion (LOQ) CUR= 25.505 ; PIP =0.56450 Accuracy%. CUR =99.36-100.586% ; PIP=98.77-100.157%	56
5	Determination of PIP in pepper (2014).	RP-HPLC method	Chromatographic Conditions: Column used: reversed-phase µBondapak CN column. Mobile phase: methanol-water flow-rate :2 ml/min Calibration curve was compared with the peak higher against PIP conc at 280-345nm	Linearity. Ranges - 10-40mg PIP 100ml methanol HPLC method is used when detection at 345 nm compared to detection at 280 nm. The method was found nearly 8 times as sensitive than HPLC separation was coupled with detection at 345 nm. So availability of a variable-wavelength detector then improves the value of the HPLC method described when compared with the traditional UV-spectrophotometric methods.	57
6	Characterization and quantification of PIP isolated from piper guineense (fam. Piperaceae (2013).	RP-HPLC method	Chromatographic Conditions. Column used: A Phenomenex C_{18} column was used. Mobile Phase: Methanol used. Flow rate o =1.40 millilitres per minute Maximum run time = 6.5 minutes and the eluent was measured at 343nm.	The calibration curve was determined to be linear over the concentration range of 0.0002 % w/v – 0.004 percent w/v. Linearity equation y = 1535x – 0.034. Coefficient of correlation, R2= 0.998. LOD and LOQ=1.872 $\times 10^{-4}$ % w/v & 5.672$\times 10^{-4}$ % w/v respectively. With RSD (percent) of 1.217 % (n=6) and 1.704 % (n=18), the approach was confirmed to be exact.	58
7	Development	RP-HPLC	Chromatographic Conditions	Linearity range (ppm): 40-100 ppm.	59

	and Validation for Estimation of PIP in Piper nigrum L(2013).	method	Column used: C_{18} column. Mobile phase - Acetonitrile, Water, and Acetic acid. Flow rate :1.0 ml/min UV detection= 340 nm Runtime =10 min	Correlation coefficient (r^2): 0.999. Regression equation=y = 41488x +11828. LOD (ppm)= 5.0 ppm. LOQ (ppm)= 5.0 ppm. Method precision (RSD %)= 0.73	
8	Application of validated method for the simultaneous estimation of CUR and PIP in Eudragit E 100 nanoparticles (2013).	RP-HPLC –PDA detection method	Chromatographic Condition: Column used: C_{18} column. Mobile phase: Combination of 0.1 % orthophosphoric acid aqueous solution and acetonitrile (45:55, v/v) in an isocratic mode elution. Flow rate = 1.2 mL min^{-1} Wavelength = 262 nm, the detection was observed	Retention time: CUR: 8.6min PIP: 5.9min. Linearity ranges: 10-150 mg mL^{-1} for both drugs. Correlation coefficients= >0.999. Limit of detection CUR: 0.3 ppm PIP: 0.1 ppm. Limit of quantification CUR :0.4 ppm PIP :0.9 ppm	60
9	Standardization of Brahmi vati - An Ayurvedic poly-herbal formulation (2013).	RP-HPLC-UV method	Chromatographic Conditions The HPLC system containing LC-20AT pump, UV detector, Rheodyne 7725 I (CA, USA) manual injector with 20 ml loop and phenomenex C_{18} column. Mobile phase: used Sodium acetate buffer and Acetonitrile. pH 3.2 adjusted with acetic acid.	Linearity range: Bacoside- 18-126 ng/ml PIP- 20-80 ng/ml. Coefficient of regression R^2 =0.998. Retention time: Bacoside- 6.83 min PIP- 9.52min. LOD value: PIP =5ng/ml Bacoside:=4/ng/ml. LOQ value: Bacoside-13.2ng/ml PIP-16.5ng/ml. Results of the recovery of Bacoside A3 and PIP ranged between 98.0 and 101.66%	61
10	Development and validation for determination of PIPE in maha-solos tablet formulation (2013).	RP-HPLC method	Chromatographic Conditions UV detector set to 343 nm. Column used: A reverse-phase Zorbax C_{18} column. Mobile phase: methanol and water as the mobile phase. Flow rate :1.0 ml/min	A good linear relationship (R^2 = 0.9997) was observed between the conc of PIPE and the peak areas in the range 0.1-0.5 mg/ml. Regression equation y = 152988146 x 293050.8667. where y is the peak area and x is the concentration of PIP. Accuracy: A recovery test was performed. recovery of PIPE was found to be in the range of 99.19-100.89 %	62
11	Simulaneous estimation of PIP and 6-gingerol in a mixture of piper nigrum and gingiber officinale (2010)	RP-HPLC method	Chromatographic Conditions Column used: A Kromasil C_{18} column was used for chromatographic separation and was detected at 280 nm. Mobile Phase (methanol:water) and detection through UV detector	Parameter's method: Stationary phase (column): Kromosil C18 Symmetry (4.6 × 250 mm) Mobile-phase: Methanol:water (70:30). Flow rate(mL/min):1.5 retention time (min) Std. 1 retention time (min)-05:000. Std.2-retention time(min)-0.3:000 Run time - 15min. HPLC method was found to be good for the simultaneous estimation of PIP and 6-gingerol in a mixture	63
12	Development and Validation for Simultaneous Determination of CUR and PIP in Human Plasma for Application in Clinical Pharmacologica l Studies (2009).	RP-HPLC method	Chromatographic Conditions Chromolith1 SpeedROD RP-18 Mobile phase: acetonitrile–methanol-trifluoroacetic acid-water (17.6:35.3: 0.1:47.0, v/v/v/v)	Ultraviolet detection: CUR:415nm PIP:335nm □-17-estradiol acetate: 280nm Linearity range:10–500 ng mL^{-1}) Linearity regression equation (i) CUR: y ¼ 0.0093 ×0.0121 (ii) PIP: y ¼ 0.0087 ×0.0095. Correlation coefficients of r^2: 0.9998 and 0.9997 for CUR and PIP respectively Flow rate =2.5 mL min^{-1} lower limit of detection of CUR and PIP was 1 ng mL^{-1}Limit of quantification was 10 ng mL^{-1}	64

TABLE 8: THE HPTLC METHODS WHICH HAVE BEEN USED FOR THE ANALYSIS OF PIP ARE SUMMARIZED IN THE TABLE BELOW

Sl. no.	Study objective	Method used	Parameters	Results	Ref.
1	A rapid method to estimate PIP in Ayurvedic formulations (2018).	HPTLC method	Chromatographic Conditions Solvent: Methanol is used as a solvent. Mobile phase= a mixture of toluene-ethyl acetate Detection at 342λ.	Linearity Range: 10-50 mg/ml. Linear regression equation: Y ¼ 100.9 + 36.16X. Regression coefficient (r^2) = 0.9999. LOD & LOQ= 20 ng and 0.228 mg/ml respectively	65
2	Estimation of PIP and piperlongumine in root of Piper longum extract and its commercial formulation (2012).	HPTLC method	Chromatographic Conditions On silica gel F_{254} HPTLC plates, the methanol extracts of the root, its formulation, and both standard solutions were applied. The plates were developed in a Twin chamber with a mobile phase of toluene: ethyl acetate and scanned at 342 and 325 nm, respectively, for PIP and piperlongumine	Instrumental precision (% RSD, n=6) PIP =0.61 piperlongumine = 0.72. Calibration range (ng/spot). PIP =20-100 piperlongumine= 30-150. Regression equation. PIP y=309.78x+801.85 piperlongumine y=3.0345x+1380.9 Correlation coefficient PIP =0.9957 piperlongumine =0.9941 Limit of detection (LOD) (ng/spot) PIP = 6.66 piperlongumine =10 Limit of quantitation (LOQ) (ng/spot) PIP= 20 piperlongumine =30	66
3	Estimation of PIP in Ayurvedic Formulation Trikatu Churna (2010).	HPTLC method	Chromatographic Conditions Stationary phase: coated silica gel G 60 F_{254} mobile phase: toluene: ethyl acetate (70:30v/v) wavelength scanning at 336 nm	Rf values: 0.42±0.03 Linearity range (ng/spot) =100-800 ng. Correlation coeificients r^2 = 0.9995 LOD & LOQ=100ng & 0.339µg respectively Recovery Studies Accuracy (%RSD) =0.353. SE =0.400. Recovery% =99.38	67
4	Method for Analysis of PIP in Fruits of Piper Species (2010).	HPTLC method	Chromatographic conditions: Solvent used: methanol is a solvent HPTLC plate coated with 0.2 mm layers of silica gel F_{254} .	Limit of detection (LOD) [ng per band] =5 Limit of Quantification (LOQ) [ng per band] =15. Linearity range =15-75ng. Correlation coefficient, r 0.994. Precision (RSD [%]). Repeatability= 0.87 Intra-day precision (n = 6) =0.97. Inter-day precision (n = 6) =0.78 Recovery [%] 94.53	68
5	Simultaneous quantification of PIP in vasavaleha (2010).	HPTLC method	Chromatographic Conditions Solvent system: The solvent system consisted of Dioxane: Toluene: Ethyl acetate: Methanol: Ammonia (1.5:2:1:1:0.3 % v/v). Densiometric analysis was carried out in the absorbance mode at 285 nm	Selection of Wavelength wavelength 254 nm is selected for further analysis. Linearity studies linear regression analysis data=0.993 regression equation Y=1237x + 1634. LOD and LOQ for PIP was found 0.5 µg/spot & 1.2 µg/spot, respectively	69
6	Quantitative estimation of PIP from the pharmaceutical dosage form (2009).	HPTLC method	Chromatographic Conditions stationary phase precoated silica gel 60F 254. Mobile phase containing Toluene and Methanol in the proportion of 80:10 v/v was used to separate the spot of PIP	RF value: 0.49±0.0 Linearity curve : 10-45mg/spot Linear regression equation : Y=327.29+153.11X Regression coefficient (r2) was 0.999.LOD and LOQ:1.23mg and 9mg, respectively	70

CONCLUSION: Several analytical methods have been developed for the detection of CUR and PIP and each method has certain advantages and limitations. The different methods used were HPLC, HPTLC, UV, UPLC, and flourimetric methods. Comparing the HPLC and UPLC methods, both provided us with a large amount of data for the analytical measurement. While comparing both the HPLC and UPLC methods, the latter provided leverage over the time, effort, and resources savings. HPTLC is a more advanced version of TLC that uses highly efficient chromatographic layers and powerful instruments for all steps of the technique, which include

accurate sample application, standardized, reproducible chromatogram creation, and software-controlled evaluation. Hyphenated techniques combine a form of chromatographic and spectroscopic techniques to take benefit of their respective advantages. Chromatography is a process that separates the different chemical components found in a mixture into pure or almost pure fractions. Comparing the Chromatographic method with the UV visible spectroscopy method, which was used to estimate CUR and PIP using methanol as a solvent simultaneously, resulted in giving the value of λmax and iso-absorptive Point of CUR and PIP at 423nm and 342 nm 368.5 nm respectively.

When the estimation was performed on simulated nasal fluid for CUR using tween 80 and sodium lauryl sulphate as a solvent, it showed an accuracy range of 99.51-100.223% and a linearity range of 5-60 µg/ml. For the characterization of CUR-loaded nano lipid carrier the solvent system that was added alongside methanol was 6.4pH phosphate buffer with 1.5% polysorbate; both the solvent systems showed comparable results. In case of the study conducted for CUR in polyherbal formulation, the solvent system was seen to be changed to ethyl acetate and that gave a linearity range of 1-5 µg/ml with a CUR of λ max 418 nm.

Estimation of CUR in nano-formulation, however again showed the incorporation of methanol as the solvent system and showed a linearity range of 5 – 25 µg/ml, no extra peak development was noticed. When CUR was estimated in poly L- lactic acid nanoparticles, the solvent system used was dichloromethane, and a LOD and LOQ of 0.05 mg/L and 0.10 mg/L were observed, respectively. From the above explanation, it is concluded that methanol is the most used solvent because both CUR and PIP dissolve in it, and it is cost-effective. Most of the CUR study done in nanoparticles system because of their low bioavailability. The studies conducted for the estimation by HPLC have shown the use of C_{18} columns as the stationary phase, where the mobile phase used was found to have acidic pH, adjusted in the range of 2-3. Some studies used phosphoric acid, and some used acetic acid to regulate the pH. The mobile phase consisted of the aqueous and organic phases, consisting of acetonitrile, methanol, and water. However, in the study involving the estimation of CUR in plasma, it was reported that acetonitrile and acetic acid was used as the mobile phase without the alcoholic part. In the co-delivered nanoparticles, the solvent system used consisted of acetonitrile and water containing SDS and disodium phosphate with acetic acid. The flow rate used in most of the studies was seen to be 0.3 ml/min to 1.3 ml/ min except in the case of the UPLC method where the flow rate was 0.4 ml/min.

Quantifying CUR in plasma and brain tissue also developed where 49% acetonitrile, 20% MeOH, 30% de-ionized water, and 1% (w/v) acetic acid were used as a mobile phase. CUR concentration reached 4–5 µg/g within 20–40 min after intraperitoneal injection R_f value of CUR: 0.48 ± 0.02. Studies conducted for the estimation of CUR by the HPTLC method were found to use silica gel 60 F_{254} as a stationary phase. Chloroform and methanol were the most common solvent other than hexane, ethyl acetate, acetone, dichloromethane and methanol for the estimation of quantification of major biomarkers in 'Mahasudarshan Churna' and Validated method for estimation of CUR in turmeric powder.

When CUR methanolic fraction of *Curcuma longa* L. rhizomes were analyzed its show linearity range 100–600 ng per spot and r^2 value 0.9998; Rf value: 0.37±0.04; LOD (ng/spot) =35; LOQ (ng/spot) = 100. The studies conducted for the estimation of CUR by Hyphenated techniques have shown the use of C_{18} columns as the stationary phase, where the mobile phase used is seen to be having more acidic pH some studies used phosphoric acid. The mobile phase consisted of the aqueous phase and organic phase, and it consisted of acetonitrile, methanol, and water for most of the time. However, in the study, estimation of curcuminoids and their metabolites in equine plasma by LC-ESI-MS/MS mixtures of two mobile phase used acetonitrile containing 0.1% (v/v) formic acid and water containing 0.1% (v/v) formic acid. Curcuminoids phase-2 metabolites, curcumin-O-sulfate and curcumin-O-glucuronide show linearity 0.5-1000 ng/mL and 1-1000 ng/mL respectively. another LC-MS/MS method for simultaneous determination of doxorubicin and CUR in polymeric micelles in subcellular compartments of MCF-7/Adrenaline cells also performed where they used 0.1% formic

acid-water and acetonitrile as a mobile phase. and result found was recovery for the two analytes was over 85% with negligible matrix effect. When all of the parameters of various UV methods are compared for estimation of PIP, the method established by U Shah *et al*. using UV VIS/ RP-HPLC techniques for the simultaneous determination of isoniazid, rifampicin, and Piperine in pharmaceutical dosage form, was found to be more accurate. In the present article, the %RSD was found to be less than 2.0% for both the methods and the R^2 value was >0.995 and the % recovery was 98-101% when the solvent system used was methanol. Similarly, the other parameters in UV methods for combined dosage drug R^2 value came out to be 0.9980 & 0.9982, LOD was 0.23 g/ml and LOQ was 0.72 & 0.69 for both the drugs i.e., curcumin and Piperine in the method developed by YB Murti *et al.*, Another method i.e. Q-Absorbance Ratio Spectrophotometric Method of two drugs rifampicin and piperine the LOD and LOQ was found to be 0.80 and 0.32 respectively. The results of the spectrophotometric method were statistically compared to those of the other UV-developed methods.

The simultaneous determination of INH, RIFA, and PIPE, UV Spectrophotometric (Absorption correction method) RP-HPLC methods were discovered to be sensitive, accurate, precise, and resilient as compared with the other developed method due to its Intra-day and inter-day deviations which have low percent RSD values (1.0 percent) that indicates that the proposed procedures are precise as compared to the other developed methods.

The studies conducted for the estimation by HPLC had shown the use of C_{18} columns as the stationary phase, except using reversed-phase µBondapak CN column when the detection was done in Piperine in pepper, quantification of Piperine isolated from piper Guineans Piperaceae, simultaneous Determination of Curcumin and Piperine in Human Plasma for Application in Clinical Pharmacological Studies respectively, where the mobile phase used is seen to be a normal condition. The mobile phase consisted of aqueous and organic phases, consisting of acetonitrile, methanol, and water for most of the time. However, in the study involving the estimation of, PIP we found the use of acetonitrile and acetic acid as the mobile phase. In the case of Eudragit E 100 nanoparticles, the solvent system used consisted of orthophosphoric acid aqueous solution and acetonitrile in an isocratic mode elution where its flow rate was found to be 1.2 ml/min and the Correlation coefficient was >0.999; therefore, the flow rate used in most of the studies was seen to be in the range of 0.8 ml/min to 2.5 ml/ min. As a whole, this method is seen to have a 98%-102% accuracy range. The studies conducted for the estimation by the HPTLC method for PIP have shown the use of silica gel 60 F_{254} as a stationary phase.

Toluene and ethyl acetate is the common solvent other than methanol which has been used in the analysis of Piperine in fruits of piper species where the LOD and LOQ were found to be 5ng per band and 15 ng per band, respectively, with linearity range of 15-75ng and the recovery % was found to have 94.53%. Apart from toluene and ethyl acetate, dioxane, methanol and ammonia in the ratio of 1.5:1:0.3 % v/v were used were LOD and LOQ were found out to be 0.5 µg/spot and 1.2 µg /spot respectively when detected in 254nm in simultaneous quantification of piperine in vasavahela (ayurvedic herbal oral formulation).

Another method has been done i.e TLC Densitometric Method for the Estimation of Piperine in Ayurvedic Formulation Trikatu Churn, which was scanned at 336 nm and its Rf value was found out to be 0.42±0.03 in the linearity range of 100-800ng, R^2 value of 0.9995 and LOD and LOQ was found to be 100 mg & 0.339 µg respectively and Recovery % was found to be 99.38 %. With the rapid evolution in the technological aspect new, improved and highly sensitive hyphenated methods have been discovered, resulting in less usage of the basic UV method. So hence we can conclude by saying that more works are being carried on by the UPLC or other hyphenated techniques rather than the UV method.

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