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Methods for the Estimation of Ellagic Acid and Curcumin in Antidiabetic Herbal Formulations – A Review

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ABSTRACT
Diabetes mellitus is the most common endocrine disorder, affecting 200 million worldwide. Despite the use of advanced synthetic drugs for the treatment, use of herbal remedies is gaining higher importance because of synthetic drugs have drawbacks and limitations. Antidiabetic herbal formulations (AHF) are considered to be more effective for the management of diabetes. Various Antidiabetic Herbal formulations are available in market which contain different constituents whose combine effect is necessary for the activity. Here, 2 constituents are selected i.e. Ellagic acid and Curcumin. An extensive survey of the literature published in various analytical and pharmaceutical chemistry related journals has been conducted and the instrumental analytical methods which were developed and used for determination of Ellagic acid and Curcumin as single or combination with other Herbal constituents in bulk drugs, formulations and biological fluids have been reviewed. This review covers the time period from 1972 to 2015 during which 48 analytical methods including spectrophotometric methods like U spectroscopy Chromatographic methods including HPLC, HPTLC and miscellaneous methods were reported.
Keywords: antidiabetic, ellagic acid, curcumin

INTRODUCTION

Diabetes mellitus is a metabolic disorder detected by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Mainly 3 types of Diabetes are frequently occur. Type I (Insulin dependent Diabetes mellitus), Type II (Non Insulin dependent Diabetes mellitus) and Gestational Diabetes. Nephropathy, Neuropathy, Cardiovascular diseases, Peripheral vascular diseases, Cerebrovascular diseases and Retinopathy are the major long term side effects observed due to the Diabetes mellitus. Currently available therapies for diabetes include insulin and various oral antidiabetic agents, which are used as monotherapy or in combination for obtaining better glycemic regulation. Many of these oral antidiabetic agents have a number of serious adverse

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effects; thus, managing diabetes without any side effects is still a challenge. Due to low toxicity and known pharmacological activity, herbal drugs have been widely and extensively used for many centuries [1].

DIFFICULTIES IN ANALYSIS OF HERBAL DRUGS

Herbal analysis is a difficult task as compared to analysis of synthetic drugs as several problems not applicable to synthetic drugs influence the quality of herbal drugs [2-7].

- Herbal drugs are combination of many components.
- The active principles are, in the majority cases mysterious.
- Selective analytical technique or reference compound could not exist commercially.
- Plant materials are chemically and naturally unpredictable.
- Chemo-varieties and chemo cultivars exist.
- Source and quality of the raw material is inconsistent.
- Adulteration and substitution is a major issue.

Different anti diabetic polyherbal formulations available in market. Different combination of the constituents are responsible for their Antidiabetic activity. Some common constituents are there which are responsible for the activity. Here, we selected some of markers i.e. Ellagic acid and Curcumin.

Drug profiles for different constituents are given in **Tables 1** & **2**.

ESTIMATION OF ELLAGIC ACID AND CURCUMIN IN SINGLE OR COMBINED FORM BY DIFFERENT ANALYTICAL TECHNIQUES

In today's world, there has been more demand for plant derived products in developed countries. These products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics. During the Nineteenth century the herbal drugs were given in form of Herbal tea mixtures, tinctures, Extracts and juices which in turn were employed in preparing medicinal drops, syrups, infusion, ointment etc., progressed of this science and because of that phytochemist succeeded in isolating active constituents, active constituents which is responsible for replacing the crude drug. Herbs are produced in two main ways: collection from wild plants from their natural habitats and cultivation of herbs that are grown that the plant collected is the one that is desired and having uniform quality attributes while in wild-crafted herbs there is a chance that the wrong herb has been picked, which could lead to serious consequences. So Herbal drugs or it's standardize extracts or pure active compound needs Analytical techniques to confirm its identity, Quality, Purity, Potency, Safety and Efficacy of the plant.

Table 1. Drug profile for Ellagic acid

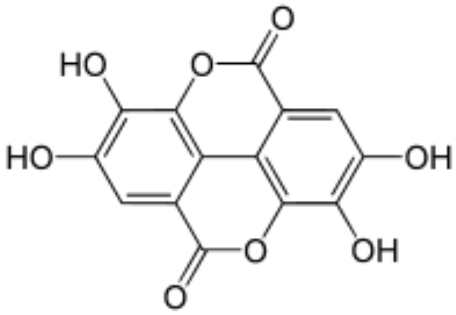
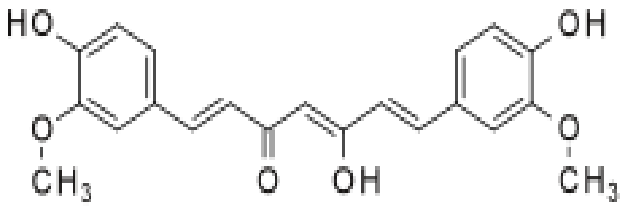
Parameter	Ellagic acid
Structure	
Synonym	2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione
Formula	$C_{14}H_6O_8$
Log P	1.59, 2.32
Solubility	Slightly soluble in alcohol; soluble in alkalies, in pyridine. Practically insoluble in ether
MP	>360°C
Density	1.67 g/cm^3
Mol. Wt.	302.197 g/mol
pKa	5.54(acidic), -4.8(basic)

Table 2. Drug profile for Curcumin

Parameter	Curcumin
Structure	
Synonym	Diferuloylmethane; curcumin I; C.I. 75300; Natural Yellow 3
Formula	$C_{21}H_{20}O$
Log P	3.29
Solubility	Insoluble in water and ether; soluble in alcohol, glacial acetic acid
MP	183 °C (361 °F; 456 K)
Mol. Wt.	368.38 g·mol^{-1}

Ellagic Acid

Table 3. HPLC

Sr. No.	Drug	Method Specification	Detection wavelength	Ref. No.
1.	Taxol and ellagic acid	**Validated HPLC method for the simultaneous determination of taxol and ellagic acid in a Punicagranatum fruit extract containing combination formulation.** • Stationary phase :- 25 x 4.6 mm, 5 µm, C18 RP (Luna) • Mobile phase :- methanol and 0.05% H3PO4, in gradient elution mode • flow rate of 1 mL/min • retention times of 13.75 min. and 11.6 min. for paclitaxel and ellagic acid, respectively	230 nm	07
2.	Ellagic Acid	**Determination of Free Ellagic Acid Content in Guava Leaves by HPLC.** • Stationary phase :- inertsil ODS-SP column (250 mm × 4.5 mm, 5µm), 35 ℃ • Mobile phase :- 3% glacial acetic acid (phase A) and net methanol (phase B) with the following gradient elution program • Injection volume:- 10µl	254 nm	08
3.	Ellagic Acid	**Determination of Ellagic Acid in Pomegranate Seeds by RP-HPLC.** • Stationary phase :- Arcus EP-C18 column (250 mm × 4.6 mm, 5 µm) • Mobile phase :- methanol and 0.1% TFA at a flow rate of 1.0 mL/min by gradient elution • flow rate of 1 mL/min	254 nm	09
4.	Ellagic acid	**Determination of ellagic acid in pseudofruits of some species of roses.** • Stationary phase :- Hypersil 200 × 4.6 mm I.D., 5 µm • Mobile phase :-mobile phase A, methanol n water n phosphoric acid (49,5:49,5:1, v/v/v), in gradient elution phases: B, methanol n water n phosphoric acid (199,5:799,5:1, v/v/v), C, methanol n water n Phosphoric acid (599,5:399,5:1, v/v/v) • flow rate of 1 mL/min • Injection volume:- 20µl	254 & 360 nm	10
5.	Ellagic acid	**Antioxidant Assay-Guided Purification and LC Determination of Ellagic Acid in Pomegranate Peel.** • Stationary phase :- TSK-gel ODS-80Tm column • Mobile phase :-2% aqueous acetic acid and methanol (gradient elution mode) • flow rate of 1 mL/min • retention times -7.7 min	254 nm	11

Table 3. HPLC (continued)

Sr. No.	Drug	Method Specification	Detection wavelength	Ref. No.
6.	Ellagic acid	**Ellagic acid content in berries: Influence of domestic processing and storage.** • Stationary phase :-LichroCART (125*3mm)RP C18, 5µm. • Mobile phase :-1% formic acid and acetonitrile in gradient elution mode. • flow rate of 1 mL/min	260 nm	12
7.	Ellagic & Gallic acid	**Development and Validation of Improved RP-HPLC method for Identification and Estimation of Ellagic and Gallic acid in Triphalachurna.** • Stationary phase :- RPHPLC C18 column • Mobile phase :-acetonitrile as solvent A and O-Phosphoric acid in Water (0.3%) as solvent B using gradients elution • flow rate of 0.8 mL/min	254 nm	13
8.	Ellagic & Gallic acid	**HPLC Analysis of Gallic and Ellagic Acids in European Oakwood (*Quercusrobur*L.) and Eucalyptus *(Eucalyptus globulus)*.** • Stationary phase :- Lichrospher RP 18 E 5µm, 10 cm • Mobile phase :-Water : Methanol: Phosphoric acid in different proportion	-	14

Table 4. HPTLC

Sr. No.	Drug	Method Specification	Detection wavelength	Ref. No.
1.	rubiadin, sennoside and ellagic acid	**Simultaneous Analysis and Quantification of Markers of *ManjisthadiChurna* Using High Performance Thin Layer Chromatography.** • Stationary phase :- TLC plates precoated with 0.2-mm layers of silica gel $60F_{254}$ • Mobile phase:- toluene:ethylacetate:methanol:formic acid (10:9:6:5 v/v) • plate was dried in hot air oven at 105° for 5 min • Scanning: Camag thin layer chromatography (TLC) scanner-III linked to Wincats software	280 nm	15
2.	Ellagic acid	**Analysis of Ellagic acid in Fresh and processed fruit products by High Performance Thin Layer Chromatography.** • Stationary phase :- TLC plates precoated with 0.2-mm layers of silica gel $60F_{254}$ • Mobile phase:- Toluene: Ethyl acetate: Formic acid= 5:5:2.5 v/v • Rf Value: 0.35	254 nm	16

Table 4. HPTLC (continued)

Sr. No.	Drug	Method Specification	Detection wavelength	Ref. No.
3.	Ellagic acid, Gallic acid and Picroside-I	**Quantification of Ellagic acid, Gallic acid and Picroside-I from *Phalatrikadikvathachurna* by HPTLC.** • Stationary phase :- TLC plates precoated with 0.2-mm layers of silica gel $60F_{254}$ • Mobile phase:- ethyl acetate-formic acidmethanol (6:0.6:0.4 v/v)	280 nm	17
4.	Gallic acid & Ellagic acid	**HPTLC Method for Estimation of Ellagic Acid and Gallic Acid in Triphalachuranam Formulations.** • Stationary phase :- TLC plates precoated with 0.2-mm layers of silica gel $60F_{254}$ • Mobile phase:-	280 nm	18
5.	Gallic acid & Ellagic acid	**Quantification of gallic acid and ellagic acid in arjunarishta by validtaedhptlc densitometry.** • Stationary phase :- 20 x 10 cm HPTLC plates coated with 0.25 mm layers of silica gel 60 F254 • Mobile phase:- toluene- ethyl acetate- formic acid-methanol, 6+6+1.2+0.25 (*v/v*) (Gallic acid) toluene-ethyl acetate-formic acid-methanol, 9+9+3+0.6 (*v/v*)(Ellagic acid) • Rf Value: 0.49±0.02 (Gallic acid) 0.46±0.02 (Ellagic acid)	290 & 285 nm	19
6.	Gallic acid & Ellagic acid	**Comparison & Quantification of Marker compound of TriphalaGuggulu by using HPTLC method.** • Stationary phase :- TLC aluminium Plates precoated with silica gel 60F254 • Mobile phase:- toluene:ethylacetate:formicacid:methanol (3:3:0.8:0.5v/v/v/v)	280 nm	20

Curcumin

Table 5. HPLC

Sr. No.	Drug	Method Specification	Detection wavelength	Ref. No.
1.	curcumin, demethoxycurcumin, and bisdemethoxycurcumin	**Improved HPLC Method for the Determination of Curcumin, Demethoxycurcumin, and Bisdemethoxycurcumin.** • Stationary phase :- C_{18} column • Mobile phase :-methanol, 2% AcOH, and acetonitrile in different proportion • flow rate of 1 mL/min	425 nm	21

Table 5. HPLC (continued)

Sr. No.	Drug	Method Specification	Detection wavelength	Ref. No.
2.	curcumin, demethoxycurcumin, and bisdemethoxycurcumin	**Development and validation of Improved Reverse Phase HPLC method for simultaneous determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin.** • Stationary phase :- C_{18} column • Mobile phase :- Acetonitrile:0.1%trifluoro acetic acid(50:50) • flow rate of 1.5 mL/min	420 nm	22
3.	curcumin and piperine	**Development and validation of simultaneous estimation method for curcumin and piperine by RP-UFLC.** • Stationary phase :-Phenomenex C8 column (250 x 4.6 mm, 5µ i.d.) • Mobile phase :-25 mM potassium dihydrogenortho phosphate buffer (pH 3.5) and acetonitrile (30: 70 v/v) • flow rate of 1 mL/min • Retention time: 4.4 min and 5.2 min for curcumin&piperine	280 nm	23
4.	curcumin and piperine	**A Liquid Chromatography Method for the Simultaneous Determination of Curcumin and Piperine in Food Products Using Diode Array Detection.** • Stationary phase :- C_{18} column (250 X 4.6 mm) • Mobile phase :-50mM potassium dihydrogen orthophosphate (pH 3.5): Acetonitrile (40:60) • flow rate of 1 mL/min	424 & 340 nm	24
5.	Curcuminoid	**Determination of Curcuminoid pigments in Turmeric by Reverse Phase High Performance Liquid Chromatography.** • Stationary phase :- Styrene Divinyl Benzene copolymer column • Mobile phase :-Acetonitrile: Water (55:45 % v/v) • flow rate of 1 mL/min • Ambient temperature	425 nm	25
6.	Sinomenine, paeoniflorin, paeonol, and curcumin	**Combinative method using HPLC quantitative and qualitative analyses for quality consistency assessment of a herbal medicinal preparation.** • Stationary phase :- Phenomenex ODS column • Mobile phase :-acetonitrile and aqueous phase (containing 0.1% phosphoric acid, adjusted with triethylamine to pH 3.5 ± 0.2) with gradient elution • flow rate of 1 mL/min	-	26

Table 5. HPLC (continued)

Sr. No.	Drug	Method Specification	Detection wavelength	Ref. No.
7.	curcumin	**A Sensitive Reversed Phase HPLC Method for the Determination of Curcumin.** • Stationary phase :- Merck C_{15} (250 cm X 4.6 mm) • Mobile phase :-acetonitril: tetrahydrofuran: 2% acetic acid 50:30:20 (2%) • flow rate of 0.7 mL/min • Retention Time: 4.587 minutes	425 nm	27
8.	Curcumin& piperine	**Application of validated RP-HPLC-PDA method for the simultaneous estimation of curcumin and piperine in Eudragit E 100 nanoparticles.** • Stationary phase :- Luna C18 column (Reversed phase, 150 mm _ 4.6 mm with 5 µm • Mobile phase :-0.1% ortho phosphoric acid aqueous solution and acetonitrile (45:55, v/v) • flow rate of 1.2 mL/min • Retention Time: curcumin at 8.685 min and piperine at 5.969 min	262 nm	28
9.	Quercetin and curcumin	**UV spectrophotometric and HPLC method development of Quercetin and curcumin in polyherbal churna and its validation.** • Stationary phase :- HiQSil C-18 column (150 mm x 4.6 mm with 5 micron) • Mobile phase :-methanol: acetonitrile: phosphate buffer (pH 5) in the ratio of 42.5 : 42.5: 15 % v/v/v • flow rate of 1.2 mL/min • Retention Time: 3.220 min. & 4.287 min. for quercetin and curcumin respectively	265nm	29
10.	curcumin, desmethoxycurcumin and bisdesmethoxycurcumin	**A simple isocratic HPLC method for the simultaneous determination of curcuminoids in commercial turmeric extracts.** • Stationary phase :- Reverse-phase chromatography on an Alltima C_{18} column • Mobile phase :-acetonitrile and 2% v/v acetic acid (40:60, v/v) • flow rate :- 2 mL/min • Retention Time: 3.220 min. & 4.287 min. for quercetin and curcumin respectively	425 nm	30
11.	Curcumin	**Stability-indicating RP-HPLC determination of Curcumin in Vicco Turmeric cream and Haridrakhandchurna.** • Stationary phase :- Lachrom HPLC with Lichrospher, ODS, (250× 4.6) mm, 5 µm • Mobile phase :-ACN: THF: 2%Aceticacid: Water (35: 30: 20:15) • flow rate :- 0.5 mL/min • Retention Time: 6.2 min	429 nm	31

Table 6. HPTLC

Sr. No.	Drug	Method Specification	Detection wavelength	Ref. No.
1.	Curcumin, dimethoxyCurcumin &bisdemethoxyCurcumin	**Extraction & Purification of curcuminoids from turmeric.** • Stationary phase :- TLC plates precoated with 0.2-mm layers of silica gel 60F_{254} • Mobile phase:- Chloroform :Methanol (95:5v/v) • Rf Value: 0.67, 0.6 and 0.506 Curcumin, Dimethoxycurcumin&Bisdemethoxycurcumin	420 nm	32
2.	Curcumin	**A HPTLC Method for chemotaxonomic evaluation of some curcuma species and their commercial samples** • Stationary phase :- TLC plates precoated with 0.2-mm layers of silica gel 60F_{254} • Mobile phase:- Chloroform: Ethanol:Acetic acid(95:4:5 v/v) • Rf Value: 0.75 for Curcumin	260 nm	33
3.	Camphor, Curcumin, dimethoxyCurcumin &bisdemethoxyCurcumin	**Comparison of *Curcuma caesia*Roxb. with other Commonly Used Curcuma Species by HPTLC** • Stationary phase :- Merck TLC plates precoated with silica gel 60 F254 (10 cm X 10 cm with 250 µm layer thickness) • Mobile phase:- toluene: ethyl acetate: methanol (18:1:1) up to 80 mm distance • Anisaldehyde sulfuric acid reagent is used as derivatizing agent for visualization • Rf Value: Camphor at 0.6, curcumin at 0.38, demethoxycurcumin at 0.3 and bis-demethoxycurcumin at 0.24	Spraying Reagent	34
4.	Curcumin and Gallic acid	**Development and Validation of HPTLC Method to Detect Curcumin and Gallic Acid in Polyherbal Microencapsulated Formulation.** • Stationary phase :- silica gel 60 F_{254} • Mobile phase:- chloroform: ethyl acetate: formic acid: methanol (7.5 mL + 6 mL + 0.5 mL + 0.5 mL) • Rf Value:-curcumin at 0.59 ± 0.02, Gallic acid at 0.25 ± 0.03	322 nm	35
5.	Circumin	**Validated method for estimation of curcumin from different varieties of curcuma longa.** • Stationary phase :- precoatedaluminium backed HPTLC plates of 0.2 mm layer thickness with silica gel 60 F_{254} • Mobile phase:- chloroform: methanol (9.5:0.5) • plate was developed up to 80 mm at temperature of 20 ± 4°C for 10 min	421 nm	36

Table 6. HPTLC (continued)

Sr. No.	Drug	Method Specification	Detection wavelength	Ref. No.
6.	Curcumin	**Validated HPTLC analysis method for quantification of variability in content of curcumin in *Curcuma longa* L (turmeric) collected from different geographical region of India.** • Stationary phase :- TLC aluminum plates precoated with silica gel 60F_{254} • Mobile phase:- toluene-chloroform-methanol (5:4:1, v/v/v) • Rf Value:- curcumin at 0.31±0.02	430 nm	37
7.	Curcumin	**Validated method for estimation of curcumin in turmeric powder.** • Stationary phase :- 0.2 mm layer thickness with silica gel 60 F_{254} • Mobile phase:- dichloromethane and methanol (99:1) • Rf Value :- curcumin at 0.43	427 nm	38
8.	Curcumin and Gallic acid	**Development and validation of HPTLC method to detect Curcumin and Gallic acid in polyherbal formulation.** • Stationary phase :- TLC aluminum plates precoated with silica gel 60 F_{254} • Mobile phase:- chloroform:ethylacetate:formic acid (7.5 mL + 6 mL + 0.5 mL) • Rf Value :- curcumin at 0.55 ± 0.02, gallic acid at 0.26 ± 0.03	254 nm	39
9.	Curcuminoids	**Improved HPTLC Method for Determination of Curcuminoids from *Curcuma longa*.** • Stationary phase :- precoated HPTLC LiChrosphere aluminium plates Si 60F_{254} • Mobile phase:- chloroform-methanol (98:2 v/v)	366 nm	40
10.	Curcumin& Ellagic acid	**Development and Validation of HPTLC Method for Estimation of Curcumin, Ellagic acid in Gel Formulation.** • Stationary phase :- silica gel 60 F254 TLC plate • Mobile phase:- toluene: ethyl acetate: methanol: formic acid (2.5: 2.5: 0.2: 0.8) • Rf Value :-curcumin at 0.6 , Ellagic acid at 0.5	348 nm	41

Table 6. HPTLC (continued)

Sr. No.	Drug	Method Specification	Detection wavelength	Ref. No.
11.	Curcumin, Metanil Yellow, and Sudan Dye	**A Simple 2-Directional High-Performance Thin-Layer Chromatographic Method for the Simultaneous Determination of Curcumin, Metanil Yellow, and Sudan Dyes in Turmeric, Chili, and Curry Powders.** **First Direction:** • Stationary phase :- silica gel 60 F254 TLC plate • Mobile phase:- chloroformmethanol (9 1, v/v) • RfValue :-curcumin (0.77), demethoxycurcumin (0.69), bis(demethoxy)curcumin (0.61), and the synthetic dye metanil yellow (0.05). **Second Direction:** • Stationary phase :- silica gel 60 F254 TLC plate • Mobile phase:- , toluene: hexane: acetic acid (50 :50 :1, v/v/v) • Rf Value :-sudan I (0.30) and sudan IV (0.23)	-	42
12.	Curcumin&Piperine	**Simultaneous Estimation of Curcumin and Piperine in Their Crude Powder Mixture and Ayurvedic Formulation Using High Performance Thin Layer Chromatography.** • Stationary phase :- TLC aluminium plates precoated with silica gel G60 F254. • Mobile phase:- Chloroform: Methanol (9.6:0.4 v/v) • Rf Value :-curcumin at 0.57 and piperine at 0.82	373 nm	43
13.	Curcumin, piperine&thymol	**Rapid HPTLC method for identification and quantification of curcumin, piperine and thymol in an ayurvedic formulation.** • Stationary phase :- silica gel 60 F_{254} plates • Mobile phase:- toluene-ethyl acetate-methanol, 9 + 1 + 0.5 (*v/v*) • Rf Value :curcumin at 0.23, piperine at 0.30, and thymol were at 0.64	420, 333 & 277 nm	44
14.	Curcumin, Piperine&Boswellic acid	**Development and validation of HPTLC method to detect curcumin, piperine, and boswellic acid in polyherbal transdermal patch.** • Stationary phase :- TLC aluminium plates precoated with silica gel $60F_{254}$ • Mobile phase:- chloroform: ethyl acetate: formic acid (7.5 mL + 6 ml + 0.2 mL) • Rf Value :curcumin at 0.48 ± 0.02, piperine at 0.52 ± 0.03, and boswellic acid at 0.61 ± 0.03)	540 nm	45
15.	Curcumin, demethoxycurcumin&bisdemethoxycurcumin	**High-performance thin layer chromatographic method for quantitative determination of curcuminoids in Curcuma longagermplasm.** • Stationary phase :- TLC aluminium plates precoated with silica gel $60F_{254}$ • Mobile phase:- chloroform: methanol (48:2, v/v) • Rf Value: curcumin, demethoxycurcumin and bisdemethoxycurcumin (R_F value of 0.66 ± 0.02, 0.48 ± 0.02 and 0.30 ± 0.02)	425 nm	46

Table 6. HPTLC (continued)

Sr. No.	Drug	Method Specification	Detection wavelength	Ref. No.
16.	Curcumin, demethoxy curcumin& bisdemetho xycurcumin	**Development of HPTLC Method and Its Validation for the Estimation of Curcuminoids from Polyherbal Mouth Ulcer Gel Formulation.** • Stationary phase :- TLC aluminium plates precoated with silica gel $60F_{254}$ • Mobile phase:- Chloroform: methanol: Glacial acetic acid (7.5: 2.0: 0.5 v/v/v) • Rf Value: curcumin at 0.56, demethoxycurcumin at 0.31 and bisdemethoxycurcumin at 0.18	430 nm	47
17.	Curcumin, demethoxy curcumin& bisdemetho xycurcumin	**Occurrence of curcuminoids in Curcuma longa: A quality standardization by HPTLC.** • Stationary phase :- TLC aluminium plates precoated with silica gel $60F_{254}$ • Mobile phase:- chloroform:methanol (48:2, v/v) • Rf Value :curcumin at 0.67, demethoxycurcumin at 0.47 and bisdemethoxycurcumin at 0.29	425 nm	48
18.	Gallic acid, Curcumin& Quercetin	**Simultaneous estimation of Gallic acid, Curcumin and Quercetin by HPTLC method.** • Stationary phase :- TLC aluminium plates precoated with silica gel $60F_{254}$ • Mobile phase:- toluene: ethyl acetate: forminc acid (4.5:3.0:0.2 *v/v/v*) • Rf Value: gallic acid at 0.40, curcumin at 0.73 and quercetin at 0.55	366 nm	49
19.	Curcumin	**Stability-indicating HPTLC determination of curcumin in bulk drug and pharmaceutical formulations.** • Stationary phase :- TLC aluminium plates precoated with silica gel $60F_{254}$ • Mobile phase:- chloroform:methanol (9.25:0.75 v/v) • Rf Value :Curcumin at 0.48 ± 0.02	430 nm	50
20.	Curcumin	**Validated HPTLC method for estimation of curcumin content in dietary supplement formulation.** • Stationary phase :- TLC aluminium plates precoated with silica gel $60F_{254}$ • Mobile phase:- n-hexane: ethyl acetate: methanol: formic acid (8: 2: 1: 2-3 drops v/v) • Rf Value :Curcumin 0.29	421 nm	51
21.	Curcumin	**Standardization of an Ayurvedic Formulation- Kalyanavleha and estimation of curcumin using HPTLC.** • Stationary phase :- TLC aluminium plates precoated with silica gel $60F_{254}$ • Mobile phase:- toluene : ethyl acetate (8:2) and toluene: ethyl acetate: methanol (9: 1: 1) as mobile phase for hexane soluble and chloroform soluble fractions respectively • Rf Value :different for different species	366 nm	52

Table 6. HPTLC (continued)

Sr. No.	Drug	Method Specification	Detection wavelength	Ref. No.
22.	Curcumin, demethoxy curcumin& bisdemetho xycurcumin	**Development and standardization of turmeric cream by HPTLC.** • Stationary phase :- TLC aluminium plates precoated with silica gel $60F_{254}$ • Mobile phase:- chloroform: ethanol: acetic acid (48:2:0.1 *v/v/v*) • Rf Value :curcumin at 0.38, demethoxycurcumin at 0.23 and bisdemethoxycurcumin at 0.16	300 nm	53

Table 7. Ultraviolet

Sr. No.	Drug	Method Specification	Detection wavelength	Ref. No.
1.	Curcumin& Quercetin	**Simultaneous estimation of Curcumin and Quercetin in Ayurvedic Proprietary Medicine by UV Spectrophotometry.** • Spectrometer: Shimazdu 1800 UV/visible soectrometer • Solvent: Methanol • λmax of Quercetin is 256 nm and λmax of Curcumin is 263 nm. • Linearity (Quercetin) : 2-20 µg/ml (Curcumin) : 4-36 µg/	256nm & 263 nm	54

Determination of Ellagic acid and Curcumin

Different analytical methods are developed for the determination of Ellagic acid alone and with other constituents. 8 HPLC and 6 HPTLC methods were developed. So, total 14 methods are used to determine Ellagic acid. Diiferent analytical methods are developed for the determination of Curcumin alone and with other constituents. 11 HPLC, 22 HPTLC, 1 UV methods were developed. So, total 34 methods are used to determine Curcumin.

CONCLUSION

The presented review highlights on various analytical methods published on Ellagic acid and curcumin, alone or in combination with other herbal constituents. HPLC method for determination of ellagic acid along with taxol was found to be most sensitive among all methods listed here. Various analytical methods like spectrophotometry, chromatography and in combinations are presented in the Tables. There is no doubt on the fact that spectroscopic methods are rapid and far more economical than chromatographic methods but their destructive nature and lack of sensitivity is huge. On the other side, estimation in Herbal formulation and impurities estimation are possible by the help of chromatography methods. Only one spectrophotometric method for the determination of curcumin was reported. In this way various analytical methods for the estimation of these Ellagic acid and curcumin in bulk or in formulation with other drugs is discussed. There are more HPLC methods are found in

the literature for the determination of ellagic acid in various fruits, while HPTLC methods are used more for the determination of ellagic acid in formulations. On the contrary, there are many more HPTLC methods are reported compared to HPLC for the determination of curcumin. The wavelength used for estimation of ellagic acid was 254 nm for HPLC and 280 nm for HPTLC in most of the reviewed literature. The presented information is useful for the researchers especially those involved in the Method development and quality control of Ellagic acid and Curcumin. This review covers the time period from 1972 to 2015 during which 48 analytical methods were developed.All Methods were found to be reproducible, accurate and can determine at Nanogram level.

FURTHER PERSPECTIVE

In Herbal Formulations, major variations are there in active constituents due to many reasonslike change in weather, change in geographical sources. Also in same place, there are observable change in amount and little change in structure of the active constituents. Due to these reasons, the effectiveness of Herbal formulation is not same all the time. To decrease these effect, exact determination of active constituents are necessary. So From the above review of Analytical methods, we can get idea of development of more specific and robust method for determination of ellagic acid and curcuminso that variation in the effectiveness of Herbal Formulations can be minimized.

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