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REVIEW ARTICLE

Citicoline: A Review of Analytical Methods

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ABSTRACT

Citicoline is a nootropic agent. It is used in the treatment of brain insufficiency and other neurological disorders such as brain stroke, trauma, and Parkinson's disease. This review article represents summarized results of published analytical methods in the literature for the determination of citicoline in biological and pharmaceutical formulations. Various techniques such as spectrophotometry, liquid chromatography (LC), high-performance LC, ultra performance LC, gas chromatography, nuclear magnetic resonance, and hydrophilic interaction LC method were reported.

Keywords: Analytical methods, citicoline, neuroprotective, nootropic

INTRODUCTION

Citicoline is a complex natural particle that functions as a moderate in the biosynthesis of phosphatidylcholine, in blood–brain barrier and achieves the central nervous system (CNS) and cognitive enhancing neuroprotective.[1-3]

Citicoline is white crystalline powder. The international nonproprietary name of citicoline is cytidine-5-diphosphocholine (CDPCho).[4] Its molecular weight is 488.32 corresponding to the molecular formula is $C_{14}H_{26}N_4O_{11}P_2$. It is soluble in water and insoluble in ethanol, chloroform, and acetone. Citicoline is a water-soluble compound with better bioavailability. Its melting point finds out between 259 and 268°C and dissociation constant (pK_a) is 4.4.[1,3-5]

CDP-choline is an intermediate in the biosynthesis of phosphatidylcholine of neuronal membranes, which increases brain metabolism and affects the levels of different neurotransmitters. Following administration, by both oral and parenteral routes, citicoline releases its two main components, cytidine, and choline.[6] Bioavailability by the oral route and intravenous route is the same. Oral choline is metabolized by intestinal bacteria to a highly volatile amine in the gut wall and liver[7] Once absorbed, choline and cytidine are re-phosphorylated, and citicoline is synthesized from cytidine triphosphate and choline monophosphate by cytidine triphosphate phosphocholine cytidylyltransferase, enter systemic circulation for usage in different biosynthesis pathways and crosses the blood–brain barrier and reaches the CNS, where it is incorporated into the membrane and microsomal phospholipid fraction. Pharmacokinetics studies show citicoline quickly retained, with less than one percent discharged in excrement.[8]

Citicoline is a safe drug, as exhibits a very low toxicity profile[7] and well-tolerated product.[8]

Citicoline has a broad spectrum of the therapeutic index with no serious side effects, as a neuroprotectant[3,9] or cerebroprotectant[1,2] neuromodulator, and as a dietary supplement. [2]

Citicoline seems valuable for enhancing both auxiliary uprightness and usefulness of the neuronal membrane that may aid membrane repair.[2] Citicoline builds the neurotransmission levels since it supports the synthesis and generation speed of dopamine in the striatum, as dopaminergic agonist through inhibition of tyrosine hydroxylase. This mechanism proposed to explain the neuroprotective activity or protective actions of citicoline in peripheral nerve injury (in CNS) have been thoroughly reviewed and include:

1. Increases norepinephrine and dopamine levels in CNS,[8,10]
2. Stimulation of phosphatidylcholine synthesis and preservation of sphingomyelin and cardiolipin levels,[6,3,10-14]

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3. Preservation of the arachidonic acid content of phosphatidylcholine and phosphatidylethanolamine by inhibiting its release and stimulating its incorporation into phospholipids,[14,15]
4. Stimulation of glutathione and glutathione reductase activity,[6]
5. Restoration of Na^+/K^+- ATPase activity,[8,10,15,16]
6. Attenuation of phospholipase A_2 activation,[10,14]
7. Attenuation of lipid peroxidation,[3,10,17]
8. Antiapoptotic effects,[3,18-20]
9. Protection of ATP levels[21] and
10. Attenuation of glutamate release.[3,21,22]

Citicoline has a neuroprotective effect in hypoxic and decreases structural cell damage caused by ischemia and improves learning memory performance in animal models of brain aging. [8] Choline precursors are exogenous agents that promote the maintenance, repair and the formation of cell membrane phospholipids as well as the neurotransmitters acetylcholine and dopamine.[9,23,24]

The pharmacological characteristic of citicoline helpful in accelerating the recovery of consciousness and motor deficit facilitates the rehabilitation of the patients with the acute ischemic cerebral vascular disease.[8,25-34] Traumatic brain injury is a major cause of death and disability.[35,36] The human amniotic fluid in models of peripheral nerve surgery,[37] reduces the infarct size and decreases mortality in hypotensive models with subarachnoid hemorrhage, and basilar artery occlusion,[38-42] decreases scarring, and promote regeneration of sciatic nerves.[43-45] Citicoline has been used for the treatment of intracerebral hemorrhage (ICH), which is a worse prognosis than the cerebral infarction.[46] The brain injury in the periphery of the hematoma is due to a vascular compression, a local inflammatory reaction, release of vasoactive substances[46-50] causes, and secondary brain injury in some models of ICH.[51,52] Citicoline translates retinal sensitivity in the long term into a protective effect on the visual field.[15,53-59] At present, glaucoma is recognized as a chronic neurodegenerative disease in which retinal ganglion cells (RGC) slowly die.[60] Citicoline acts positively on the glaucomatous optic nerve damage with the enhancement of PtdCho synthesis to counteract neuronal apoptosis and protect RGC from degeneration.[55,61] Intramuscular citicoline appeared to improve visual acuity, contrast sensitivity, and visual-evoked potentials,[15,55,62] and in a double-blind placebo-controlled trial[58] such treatment led to the improved function of both retinal and post-retinal visual pathways.

Moreover, citicoline has been effective in senile cognitive impairment, nerve regeneration[63] and scarringinmodels,[64]chroniccerebralischemia,[28,65-67] improve deficit, stroke rehabilitation, cerebrum and spinal injury, neurological disease, and in amblyopia and glaucoma.[53,68] CDP-choline has been reported to decrease infarct volume and edema to improve neurological deficits.[26,44,69] Citicoline accelerates phospholipid synthesis, neural repair,[24] also as epilepsy,[70] Alzheimer's disease,[71,72] Huntington's disease,[73] Parkinson's disease, and amyotrophic lateral sclerosis.[74,75]

SOLUBILITY

According to Biopharmaceutics Classification System (BCS), classification of Citicoline falls under BCS Class I, meaning it has high solubility and high permeability.[1,76] The solubility of the drug was tested in solvents routinely used for the analytical methodology.

Sample preparation is an integral part of the analytical methodology. Figure 2 shows various diluents used for the analysis of citicoline. In reported articles, majorly the aqueous solution of the citicoline is used for the analytical procedures.

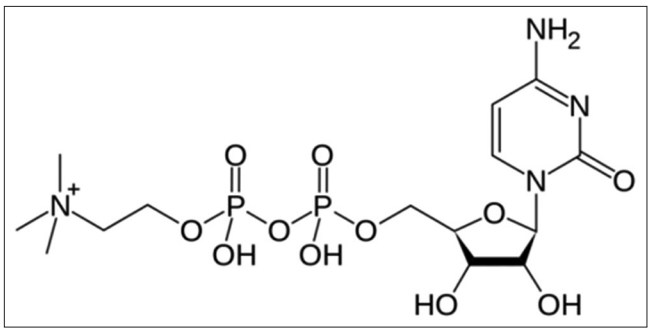

Figure 1: Structure of citicoline

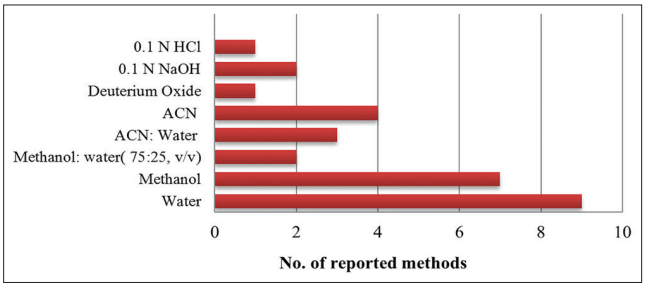

Figure 2: Various diluents used for the analysis of citicoline

ANALYTICAL METHODS

Ultraviolet (UV) spectrophotometric method

In the literature survey, eight methods were reported for the estimation of Citicoline (Citi) using spectrophotometry, of which three methods are for determining Citi alone, while the others are for quantifying Citi in combination with other drug substances. Table 1 shows, the summary of the reported spectrophotometric methods indicating the basic principle method, wavelength maxima, and limit of detection (LOD).

Prajapati *et al*.[77] developed two methods for simultaneous determination of Citi and Pira in fixed-dose combination products, Method-1: Absorption correction method and Method-2: Q-absorbance method. For Method-1: Wavelengths selected for Citi was 266 nm as Pira shows zero absorbance. Hence, the absorbance of Pira was found by subtracting the absorbance of Citi. Absorbance corrected for Pira was measured at 226.5 nm Method-2: Using wavelength 220 nm (λ_{max} of Pira) and 228 nm (iso absorptive point). This method obeyed Beer's law in the concentration range of mixture of 10–50 µg/ml for Citi and 100–500 µg/ml for Pira. The results of analysis have been validated.

A spectroscopic method was developed by Patel *et al*.[78] for simultaneous estimation of EDA and CitiS in the synthetic mixture using ratio derivative method. The ratio was taken using divisor, for 1–6 µg/ml EDA was 25 µg/ml of CitiS, and for CitiS 25–150 µg/ml was 6 µg/ml of EDA. After divisor spectra were converted to the second derivative. EDA showed zero crossing point at 258.40 nm while CitiS showed zero crossing point at 267 nm. The absorbance was measured at 267 nm for EDA and 258.40 nm for CitiS and calibration curves were plotted as absorbance versus concentration, respectively. The linear correlation was obtained in the range of 25–150 µg/ml for CitiS at 258.40 nm. The method was successfully applied for simultaneous determination of EDA and CitiS in the binary mixture.

Sivadas *et al*.[79] reported the methods for simultaneous determination of Citi and Pira in the combined dosage form. The first method was developed by simultaneous equations using 280.3 and 264.1 nm as two analytical wavelengths. The second method was the absorbance ratio in which wavelengths selected were 256.6 nm as its absorptive point and 280.3 nm as λ_{max} of Citi. Both the drugs obeyed Beer-Lambert's law at the selected wavelength in the concentration range of 5–13 µg/ml for Citi and 10–22 µg/ml for Pira. According to the International Conference on Harmonization (ICH) norm, the methods were validated statistically and by recovery studies.

UV spectrophotometric method as developed by Pathan *et al*.[80] for the simultaneous estimation of Citi and Pira from the bulk and tablet formulation. The λ_{max} of Citi and Pira were found at 270 nm and 220 nm, respectively, with water. For the absorbance correction method, Pira was determined using specific absorbance at 220 nm, where the absorbance of Citi was subtracted from the total absorbance. At selected wavelengths the linear concentration range of 50–150 µg/ml for Citi and 100–300 µg/ml for Pira. The developed and validated method was applied for analysis of drug content from tablet formulation.

Patel *et al*.[81] developed a derivative spectrophotometric method to quantify CitiS

Table 1: Spectrophotometric methods for the analysis of citicoline

Compounds	Method	Amax	Solvent/procedure	LOD (µg/ml)	Ref.
Citi, Pira	Absorption correction method Q-absorbance method	266, 226.5 228,220	Water + 0.1 N NaOH	0.269, 7.124	[77]
CitiS, EDA	Ratio derivative method	267, 258.4	Distilled water	0.195, 0.034	[78]
Citi, Pira	Method A Method B	280.3, 264.1 256.6	0.1 N Methanolic HCl	-	[79]
Citi, Pira	Absorption correction method	270, 220	Distilled water	-	[80]
CitiS	-First-order derivative	272 286	Distilled water	0.75 0.69	[81]
CitiS, Pira	Second-order derivative method absorbance correction method	274.6 206.8	Distilled water	0.74 0.40, 0.42	[82]
Citi	Calibration curve method	272	Distilled water	0.49	[83]
CitiS	Difference Spectrophotometric method	239 (maxima), 283 (minima)	0.1 M NaOH, 0.1 M HCl	-	[84]

LOD: Limit of detection

used as a single active principle in pharmaceutical dosage forms. CitiS shows spectrophotometric characteristic at 272 nm for simple UV spectrum and 286 nm for derivative spectrum (1D286) was found adequate for quantification. The linearity signal and concentration of CitiS in the range of 10–50 µg/ml in aqueous solution present a correlation coefficient of 0.999 for simple UV and 0.9997 for first-order derivative spectrum.

A method has been developed and validated by Dhoru *et al*.[82] for the simultaneous estimation of CitiS and Pira in pharmaceutical formulation by the second-order derivative method for Citi and absorbance correction method for Pira. For the second-order derivative method, the wavelength selected was 274.60 nm for estimation of Citi. For absorbance correction method, the wavelength selected was 206.8 nm for estimation of Citi and Pira. Both drugs follow Beer-Lambert's law over the concentration range of 5–50 µg/ml for Citi and 4–28 µg/ml for Pira. The proposed method applied for the determination of Citi and Pira in the combined dosage form.

Sachan *et al*.[83] developed a method for the determination of Citi using double beam UV spectrophotometer. Citi has absorption maxima at 272 nm, and the measurements were obtained against distilled water. The Beer Lambert's law was obeyed in the concentration range of 5–50 µg/ml with correlation coefficient 0.9998. It's further implemented for the quantification of the active compound in the pharmaceutical specialty for quality control.

Panda *et al*.[84] reported a spectrophotometric method for the estimation of CitiS in tablets. This spectrophotometric method is based on the principle that CitiS shows two different forms that differ in the absorption spectra in the basic and acidic medium. Solvents used were 0.1 M NaOH and 0.1 M HCl. The maxima and minima in the difference spectra of CitiS were found to be 239 nm and 283 nm, respectively. Amplitude was calculated from the maxima and minima of the spectrum. The drug follows linearity in the range of 1–50 µg/ml (R^2 = 0.999). Validation of the proposed method has been performed.

Chromatography

High-performance liquid chromatography (HPLC)

Biological samples

Various methods for the determination of citicoline in biological samples such as plasma and serum are listed in Table 2.

1. Bindaiya *et al*.[85] developed a high-performance reversed-phase liquid chromatographic (RP-LC) method for measurement of Citi monosodium in human plasma. The active drug was isocratically eluted at a flow rate of 1 ml/min at ambient temperature in a nucleosil C_{18} analytical column with a mobile phase composed of tetrabutylammonium hydrogen sulfate buffer (0.005 M, pH 5.0):methanol (95:05, v/v). Photodiode array (PDA) was performed at 270 nm and the retention time of the drug was observed to be 6.64 min. The method was found to be linear in the drug (Citi monosodium in spiked plasma) concentration range 150–900 ng/ml. The method was validated, and the drug is stable in human plasma under various test conditions, and the proposed method can be effectively utilized for investigation of CTM in human plasma and pharmacokinetic studies.
2. A HPLC method was developed by Chen *et al*.[86] to determine uridine, metabolite of Citi, in human plasma, and applied to the pharmacokinetics and bioequivalence studies of CitiS tablet and capsules. The separation was performed on a Phenomenex kinetex C_{18} (100 mm × 4.6 mm, 2.6 µm) column,

Table 2: HPLC methods to determine CITI in biological samples

Matrix	Compound	Mobile phase	Column	Detection	Amax	Flow rate	LOD	Ref.
Human plasma	Determine of CitiM	TBAHS buffer (0.005 M: methanol (95:05 v/v)	Nucleosil C18 column	UV	270 nm	1 ml/min	20 ng/ml	[85]
Human plasma	Pharmacokinetics and bioequivalence studies	0.05 mol/L phosphate buffer: methanol (98:2 v/v)	Phenomenex kinetex C18 (×100 4.6 mm, 2.6 µm)	UV	260	0.8	-	[86]

Citi*: Citicoli, CitiS*: Citicoline Sodium, Pira*: Piracetam, EDA*: Edaravone, CitiM: Citicoline monosodium. UV: Ultraviolet, LOD: Limit of detection, HPLC: High-performance liquid chromatography

Table 3: Reported analytical HPLC methods for determination of Citi with other drugs

Study Aim	Mobile phase	Column	Detection	λmax	Flow rate	LOD	Ref.
In pharmaceutical preparations	Tetra butyl ammonium hydrogen sulfate buffer (pH 6):Methanol (95:05, v/v)	C18 column (250 mm × 4.6 mm ×, 5 µm particle size)	UV	270	1 ml/min		[87]
Simultaneous estimation with MCB	A mixture of buffer (pH 6, adjusted with Orthophosphoric acid):methanol (50:50 v/v)	Agilent Zorbax C18 (150 mm, 4.6 and 3.5 µm) column	UV	257	1	2.96,2.8	[88]
Stability indicating assay	Water: orthophosphoric acid (99:1 v/v)	Phenomenox C18 column (250 mm × 4.6 mm)	UV	272	1	-	[89]
In pharmaceutical dosage form	Buffer (potassium dihydrogen phosphate and tetra butyl ammonium hydroxide):methanol (95:05 v/v)	BDS Hypersil C18 column (250 mm 4.6 mm and 5 µm)	UV	276	0.8, 1.0,1.2	-	[90]
In pharmaceutical dosage form using an IS method	Acetonitrile: phosphate buffer (pH 5.0) (55:45 v/v)	RP C18 Phenomenex (250 mm × 4.6 mm and 5 µm) column	UV	270	1.0	0.534	[91]
In bulk and pharmaceutical dosage form	Acetonitrile: 0.02M KHPO (60:40 v/v)	Phenomenex Luna C18 column (250 mm × 4.6 mm and 5 µm particle size)	UV	554	1	0.03	[92]
In pharmaceutical dosage form using an IS method	Acetonitrile: water (20:80, v/v) (adjusted pH 3.0 using orthophosphoric acid)	RP C18 column (250 mm × 4.6 mm and 5 µm)	UV	260	0.7	1.4	[93]
Stability indicating method	Buffer: methanol (100:2)	Waters 2487 Hypersil BDS C18 (250 mm × 4.6 mm) 5 µ column	UV	280	1	-	[94]
Stability Indicating Method in Injection formulation	Phosphate buffer: methanol (95:5%v/v)	Cosmosil C18 (250 mm × 4.6 mm and 5 µm)	UV	276	1	-	[95]
Simultaneous estimation with methylcobalamin	Phosphate buffer: ACN (50:50)	Insertion C18 YMC (250 mm × 4.6 mm and 5 µm)	UV	295	0.8	500-1500	[96]
Estimation of Citi sodium in bulk and dosage form	Potassium dihydrogen phosphate buffer: ACN (30:70)	Phenomenex Luna C18 (250 mm × 4.6 mm and 10 µm)	UV	272	1	-	[97]
Simultaneous estimation in the liquid oral formulation	0.1 M monobasic potassium phosphate: methanol (70:30, v/v)	Merck C8 column (250 mm × 4.6 mm and 5 µm particle size)	UV	294	1.5	-	[98]

Citi*: Citicoli, CitiS*: Citicoline Sodium, Pira*: Piracetam, EDA*: Edaravone, CitiM: Citicoline monosodium. UV: Ultraviolet, LOD: Limit of detection, HPLC: High-performance liquid chromatography

with a mobile phase of 0.05 M phosphate buffer (potassium dihydrogen phosphate, balanced pH to 3.5 by phosphate):methanol (98:2, V/V), and delivered at the flow rate of 0.8 ml/min. The calibration curve was linear over uridine concentration of 0.05–2 µg/ml. The fundamental pharmacokinetic parameters ($t_{1/2}$ T_{max} C_{max} AUC) and relative bioavailability of CitiS tablet and capsule were observed. The tablet and capsules were bioequivalence.

Pharmaceutical samples

Analytical methods for the determination of Citi in pharmaceutical dosage forms using HPLC show in Table 3, the best HPLC methods for the analysis of Citi.

1. Bindaiya *et al*.[87] gave an HPLC method for the quantitative estimation of CitiM in a pharmaceutical preparation. Isocratic separation was utilizing C_{18} column (250 mn × 4.6 mm and 5 µm) with a mobile phase consisting of tetra butyl ammonium hydrogen

sulfate buffer (pH 6):methanol (95:05, v/v), the flow rate was 1.0 ml/min, and detection wavelength was 270 nm. The proposed method has the linearity range of 20–100 μg/ml with a coefficient of correlation 0.9999. The column was kept up at ambient temperature and analytical run time of approximately 10 min, and it was eluting at roughly 6.3 ± 0.5 min. The proposed method was validated.

2. A reversed-phase-HPLC (RP-HPLC) method was reported by Kavitha *et al*.[88] for simultaneous estimation of Citi and methylcobalamin. Chromatographic separation was performed on Agilent Zorbax C_{18} (150 mm, 4.6 mm, and 3.5 μm) column, with a mobile phase comprising the mixture of buffer (pH 6.0, balanced with orthophosphoric acid) and methanol in the proportion of 50:50 v/v, at the flow rate 1 ml/min. The detection was carried out at 257 nm. The retention time of Citi and methylcobalamin was observed to be 1.8 and 3.9 min, respectively. The linearity of Citi was found in the range of 50–150 μg/ml with correlation coefficient 0.999.
3. Patel and Prajapati[89] have discussed stability-indicating an HPLC method for analysis of drug in the presence of degradation products. Degradation was found to occur in hydrolytic, oxidative, and thermal condition, while the drug was stable to photolytic and humid conditions. The proposed chromatographic method was fine using the sample generated from forced degradation studies. Good resolution between the peaks corresponds to degradation products, and analyte was achieved on a C_{18} column, mobile phase consists of a mixture of water: orthophosphoric acid (99:1, v/v) (pH 4.0). Quantitation was achieved with UV detection at 272 nm, and the method was validated.
4. An analytical RP-HPLC method was reported by Sharma and Chand[90] estimation of CitiS. The chromatography was carried out isocratically by a BDS Hypersil C_{18} column (250 mm × 4.6 mm and 5 μm) with a mixture of buffer (potassium dihydrogen phosphate and tetra butyl ammonium hydroxide): methanol (95:05 v/v) as a mobile phase. Detection was carried out utilizing a UV detector at 276 nm wavelength. The retention time was observed to be 4.83 min. System suitability parameters such as tailing factor 1.19, theoretical plate 5397, and injection precision 0.030506% for n = 5 were calculated. The developed method was observed to be linear (0.9999) in the concentration range of 80–180 μg/ml for CitiS.
5. Sandhya *et al*.[91] proposed an RP-HPLC method for the determination of CitiS utilizing pyrimethamine as internal standard (IS). The HPLC instrument utilized was Shimadzu LC-20AD with turnaround stage C_{18} phenomenex (250 mm × 4.6 mm and 5 μm) column utilizing acetonitrile: phosphate support at pH 5.0 (55:45 v/v) as a portable stage. The flow rate was kept up at 1.0 ml/min, and UV detection was carried out at 270 nm. The method was validated as indicated by ICH guidelines. The retention time was observed to be 2.26 ± 0.03 min for CitiS and 4.46 ± 0.025 min for IS. The regression analysis demonstrated great recovery over the concentration range of 5–25 μg/ml for CitiS. The recovery studies of the method give great recovery in the range of 99.89–100.48% with under 2% of relative standard deviation (RSD).
6. RP-HPLC method has been developed and validated by Singh *et al*.[92] for the estimation of CitiS with methylcobalamin. The partition was carried out using Phenomenex Luna C_{18} (250 mm × 4.6 mm, 5 μm) in isocratic mode, with a mobile phase containing acetonitrile:0.02M KH2PO4 (60:40, v/v). The flow rate is 1 ml/min, and effluents are observed at 554 nm. Chromatogram showed a peak at a retention time of 3.8 min for CitiS and 2.3 min for methylcobalamin. The proposed method is validated and can be effectively applied for the quantitative determination of CitiS with methylcobalamin.
7. A RP-HPLC method was developed by Surani *et al*.[93] for determination of CitiS. Chromatographic separation was carried out on a C_{18} column utilizing a mobile phase comprising acetonitrile:water (20:80, v/v) balanced at pH 3.0 utilizing 1% orthophosphoric corrosive. The flow rate was kept up at 0.7 ml/min, and UV identification was carried out at 260 nm. Caffeine was utilized as an IS. The calibration curve was observed to be linear over the range 1–500

μg/ml. The strategy was observed to be basic, fast, and simple to apply, making it extremely appropriate for routine examination of CitiS in pharmaceutical dose frame.

8. Uttarwar *et al*.[94] developed a RP-LC method for the quantitative determination of Citi from Citi sustained release tablet. The chromatographic separation was accomplished on Waters 2487 Hypersil BDS C_{18} (250 mm × 4.6 mm and 5 μm) column, and the mobile phase containing Buffer:methanol (100:2). The flow rate was 1.0 ml/min and the detection wavelength was 280 nm, and the injection volume was 20 μl. The method was validated, and the tablet was subjected to stress condition of hydrolysis, heat degradation, acid degradation, and base degradation. No interference of degradation products was found at the retention time of standard principle.
9. A novel stability indicating LC method was developed and validated by Ganduri *et al*.[95] for quantitative estimation of CitiS in injection formulation in the presence of degradation products generated from forced degradation studies. An isocratic RP-LC technique was developed to isolate the drug from the degradation products utilizing Cosmosil C_{18} (250 mm × 4.6 mm and 5 μm) column and a mobile phase constituted of phosphate support and methanol (95:5% v/v). The wavelength of the detection is 276 nm. Citi was subjected to stress condition of hydrolysis (acid and base), oxidation, photolysis, and thermal degradation.The validation data demonstrated that the assay is sensitive, specific and reproducible for the determination of CitiS in injection formulation in the presence of stress degradants and impurities. The technique is linear from 125 μg/ml to 375 μg/ml. The accuracy of the method was observed to be 98.30%.
10. A RP-HPLC technique has been developed and validated by Dara *et al*.[96] for the simultaneous estimation of Citi and methylcobalamin in combination. The separation was completed utilizing a mobile phase comprising phosphate buffer and acetonitrile in the proportion of 50:50. The pH of the mobile phase was adjusted to 3.0 with orthophosphoric acid. The column utilized was Inertsil C_{18} YMC (250 mm × 4.6 mm, 5 μm) with the flow rate of 0.8 ml/min utilizing UV detection at 295 nm. The total run time was 8 min and the retention time of Citi and methylcobalamine was 3.2 min and 4.1 min, respectively. The proposed method was linear for the assay of Citi and methylcobalamine over a concentration range of 500–1000 μg/ml and 0.75–2.25 μg/ml, respectively. Results of the analysis have been approved statistically and by recovery studies.
11. Maradiya and Pansara[97] reported a reversed-phase HPLC method for the determination of CitiS. The drug was resolved on a C_{18} column Phenomenex Luna, (250 mm × 4.6 mm and 10 μm), using mobile phase of potassium dihydrogen phosphate buffer and acetonitrile (30:70). The mobile phase was delivered at the flow rate of 1.0 ml/min and detection was done at 272 nm. Separation was finished inside 2.22 min a calibration curve was linear with great relationship coefficient (0.999) over a concentration range of 10–60 μg/ml. The developed method was applied to the determination of the CitiS and for performing stability studies.
12. A RP-HPLC method has been reported by Borkar *et al*.[98] for simultaneous estimation of Citi and preservative methylparaben (MetP) in oral drop formulation. Chromatographic separation was completed on (Merck) C_8 column (250 mm × 4.6 mm and 5 μm) utilizing a mobile phase consisting of 0.1 M monobasic potassium phosphate:methanol (70:30, v/v). The flow rate was kept up at 1.5 ml/min and 30°C section temperature with the detection wavelength at 294 nm. The calibration curve was observed to be linear over the concentration range of 80–120 ppm for CitiS and 8–12 ppm for MetP with a correlation coefficient of 0.999 and 0.999 for Citi and MetP, respectively. The maintenance times were observed to be 2.06 and 14.68 min for Citi and MetP, respectively.

RP-ultra fast liquid chromatography (UFLC)

Panda *et al*.[99] developed a simple, precise, and accurate RP-UFLC method for simultaneous determination of CitiS and Pira in tablets. Separation was done on an Enable C_{18} G column (250 mm × 4.6 mm and 5 μm) using methanol:water (10 mm TEA) (75:25, v/v) as mobile phase at the flow rate of 1 ml/min in isocratic mode. The

PDA detection wavelength was 225 nm. The retention time of CitiS and Pira was 1.985 and 3.007 min, respectively. The method was linear in the concentration range of 1.0–250 μg/ml with correlation coefficients of 0.999 for both the drugs. The LOD and limit of quantification (LOQ) for CitiS were 0.25 μg/ml and 0.81 μg/ml, respectively. The average recoveries for recovery study were observed to be in the range of 100.24–101.09% and 100.08–101.05% for CitiS and Pira, respectively. The RSD values for intraday, interday, and system precision were observed to be <2%. The method was applied successfully for simultaneous estimation of CitiS and Pira in combined tablet formulation.

Ultra-performance liquid chromatography

Alagar *et al*.[100] been developed and validated for the determination of CitiS and Pira in its pharmaceutical dosage form. Chromatographic separation was achieved on a beh shield C_{18} (2.1 mm × 100 mm and 1.7 μm), by a mobile phase consisting of water: acetonitrile ratio (45:55 v/v, pH 2.8) with a flow rate of 0.3 ml/min. The detection wavelength was set at 225 nm. Citi and Pira were subjected to different stress conditions. The method was linear (0.999) at a concentration range of 5–25 μg/ml. The intra and interday precisions were satisfactory did not exceed 2%. Moreover, the accuracy of the method was proved the mean recovery of CitiS and Pira was 99.04–101.58%. The proposed method has high throughput as the analysis involved short run-time (3 min). The technique met the ICH/Food and Drug Administration regulatory requirements. The results showed that the method can be applied successfully for routine use in quality control industry laboratories.

Gas chromatography (GC)

Kempegowda *et al*.[101] reported that the active pharmaceutical ingredients are an ingredient in a pharmaceutical drug that is biologically active which are basically drug substance and give therapeutic benefit. There is a process solvent which incorporates methanol and isopropyl alcohol (IPA) which are fundamentally organic volatile impurities (OVI) used during the manufacturing process. The developed method was linear with a correlation coefficient (0.999) in the concentration range of 1–150% solutions containing methanol, IPA at a concentration. To identify and control these solvents GC - headspace method was developed. The method was validated as per the ICH guidelines and United States Pharmacopoeia. The percentage RSD for LOD and LOQ for OVI (i.e. methanol and IPA) were 22.3 and 45.2 and 104.9 and 125.1, respectively.

Hydrophilic interaction liquid chromatography (HILIC) method

Derbouz *et al*.[102] detailed that a stability-indicating HILIC method was developed for analysis of Citi in the presence of its degradation products. It was performed utilizing Atlantis HILIC Si column (50 mm × 4.6 mm and 3 μm) and a mobile phase constituted by 70% of acetonitrile in formate buffer 0.02 M (v/v) adjusted to pH 3. The column's thermostat compartment was adjusted at 30°C. Citi was subjected to oxidative, acidic, basic, hydrolytic, thermal, and UV light stress conditions. The column effluents were monitored at 270 nm by PDA and mass detectors. Depending on the stress, three degradation products showed up: One after the acidic or photolytic action, the second by NaOH reaction, and the third with oxidative stress. LC– ESI–MS/TOF, ^{1}H, and ^{13}C nuclear magnetic resonance were used to characterize them. The most probable development systems of the identified impurities were proposed. The developed method was validated in terms of specificity, LOD and quantification, linearity, accuracy, and precision as per the ICH guidelines. The proposed method was successfully applied for the determination of Citi in dosage forms.

DISCUSSION

As discussed, citicoline belongs to BCS Class-I, and it is soluble in water. Selection of diluents would not be problematic in the analysis of citicoline as there are concerns with various compositions of aqueous organic phase. The systemic review covers the current analytical methods for the estimation of citicoline and its combination in the pharmaceutical and biological sample. For spectrometric determination, complexity with dosage forms includes the presence of multiple entities and excipients, which may cause a

considerable challenge in the analytical testing during the development of assay procedure. Estimation of individual drugs in the multi dosage forms preferred HPLC methods. Furthermore, the run times are prolonged with greater tailing factor in HPLC.

CONCLUSION

A broad range of techniques is available for the analysis of citicoline in pharmaceutical formulations and biological samples. The analysis of the reported data revealed that the HPLC was extensively used for the determination of citicoline in biological samples like plasma. For analysis of citicoline in pharmaceuticals, HPLC with UV is applicable because this method provides accurate, precise, and low cost compared to more advanced detection techniques. The review carried out an overview of the current state-of-the-art analytical methods for estimation of citicoline.

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