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Review Article

Nootropic drugs: Piracetam and Citicoline sodium: A Review of Analytical Methods

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Abstract:

Piracetam and Citicoline sodium are two nootropic drugs or psychostimulants. They are used to improve cognitive impairment in patients with brain injury. Their combined dosage forms help enhance cognition and memory, as well as slow the progression of brain aging. Increasing blood flow and oxygen to the brain aids stroke recovery and improves Alzheimer's, Down syndrome, dementia and dyslexia.

This review article provides an extensive computer-assisted survey of the literature on analytical techniques developed for the quantification of Piracetam and Citicoline in bulk, pharmaceutical formulations, and biological fluids. Bioanalytical methods are essential for the accurate measurement of drugs and their metabolites in biological matrices. Several analytical approaches, as spectrophotometric, chromatographic and electrochemical methods have been widely applied for the quantitative analysis of these drugs in both biological specimens and pharmaceutical products. Thus, the primary aim of this review is to summarize and highlight the current analytical methodologies used for the assay of Piracetam and Citicoline sodium.

Key words:
Piracetam, Citicoline sodium, Psychostimulants, Analytical methods.

Highlights

- Piracetam and Citicoline sodium are nootropic agents used to improve cognitive function and treat brain-related disorders.
- These drugs are beneficial in managing stroke, Alzheimer's, dementia, Down syndrome, and dyslexia.
- The review covers a wide range of analytical techniques for their quantification in bulk materials, dosage forms, and biological fluids.
- Spectrophotometric, chromatographic, and electrochemical methods are the primary analytical tools reviewed.
- It is considered to be the first review article that summarizes the analytical methods for the determination of both Piracetam and Citicoline sodium.

1. Introduction

Nootropic drugs—also referred to as cognitive enhancers, neuro-enhancers, or more recently, "smart drugs" were originally developed to treat elderly psychiatric patients, with a primary focus on improving memory.[1, 2] Today, however, the term is more commonly associated with substances that enhance brain functions such as memory, attention, and concentration in healthy individuals.[1, 2] Because of their appealing cognitive benefits and the perception of minimal serious side effects, these substances have become increasingly popular for non-medical use. They are often used as over-the-counter "smart drugs" especially to boost academic performance and manage the stress of daily life.[1,3] Studies indicate that the use of cognitive enhancers among European students increased to 16%, while in the United States, usage rates have been reported as high as 35%.[4,5] Despite the non-medical use of these compounds posing serious concerns about health, legality, and ethics, there has been a remarkable surge in the manufacturing of nootropic medicines over the last decades.[6] Piracetam (PIR), scientifically named 2-(2-Oxopyrrolidin-1-yl)acetamide (**Fig.1a**)[7], is a cyclic derivative of the neurotransmitter gamma-aminobutyric acid (GABA), used as a cognitive-enhancing compound that helps protect the brain's cerebral cortex from oxygen deficiency (hypoxia). Giurgea developed it in 1964 and later categorized it as a nootropic.[8] PIR is a white or almost white powder, which is freely soluble in water and soluble in ethanol. Its molecular weight is 142.2 g/mol, corresponding to the molecular formula $C_6H_{10}N_2O_2$.[7] Its melting point is 152 °C, and its dissociation constant (pKa) is 15.67.[9] It exerts its effects by enhancing the function of

various neurotransmitter systems, such as the cholinergic, dopaminergic, and noradrenergic pathways, while also supporting the stability of neuronal receptors. It offers neuroprotection by shielding neurons from harmful substances and aiding in the recovery of impaired neurotransmission.[8] Citicoline sodium (CIT) is chemically identified as the monosodium salt of Cytidine 5'-(trihydrogen diphosphate), P'-[2-(trimethylammonio)ethyl] ester, in its inner salt form (**Fig.1b**).[10] CIT is categorized as a psychostimulant and nootropic drug used to treat neurological conditions such as Parkinson's disease, Alzheimer's disease, brain stroke, and trauma, as well as brain insufficiency.[11] It is a white crystalline powder, highly soluble in water, and insoluble in ethanol, chloroform, and acetone. Its molecular weight is 510.31g/mol, corresponding to the molecular formula $C_{14}H_{25}N_4NaO_{11}P$.[10] Its melting point is between (25.9 – 26.8) °C, and its dissociation constant (pKa) is 4.4.[11] CIT supports the cholinergic system by supplying choline to boost acetylcholine synthesis.[12] It also provides neuroprotection by increasing central levels of dopamine and norepinephrine, enhancing phospholipid production, and maintaining vital membrane lipids such as sphingomyelin and cardiolipin. It also boosts antioxidant defenses by activating glutathione systems, restores Na^+/K^+-ATPase function, reduces lipid peroxidation and phospholipase A2 activity, and further protects neurons by preserving ATP, limiting glutamate release, and preventing apoptosis.[4]

In the pharmaceutical field, analytical methods are essential techniques used to analyze the chemical, physical, and biological properties of drugs, their ingredients (active pharmaceutical ingredients and excipients), as well as related substances, from development to manufacturing, quality control, and post-market surveillance.[13]

Pharmaceutical analytical methods encompass a wide range of techniques, often categorized into three main types: spectrophotometric methods, chromatographic and electrochemical methods. The spectroscopic techniques are the methods that measure the interaction of electromagnetic radiation with matter to provide information about structure and concentration. Mainly in quantitative methods of analysis, UV-Visible (UV-Vis) Spectrophotometry is used, which measures the absorption of UV or visible light, commonly used for quantification of drugs and dissolution testing.[14] Spectrofluorimetry

is a sensitive analytical technique that measures the fluorescence emitted by substances when excited by light, usually in the UV or visible range. It is widely used for detecting and quantifying fluorescent compounds in fields like pharmaceuticals, biochemistry, and environmental science. The method is highly sensitive, capable of detecting very low concentrations, and is especially useful for studying drug interactions and protein binding.[15] Regarding chromatographic techniques, these methods are used for separating, identifying, and quantifying components in mixtures and their pure forms. As examples, Gas Chromatography (GC) is primarily used for volatile compounds or for compounds that must be thermally stable and can be derivatized to be volatile. As usual the GC technique requires a mobile and a stationary phase. The mobile phase is a chemically inert carrier gas, for example, argon, helium, or nitrogen. The mobile phase carries the analyte molecules through a heated column. The stationary phase consists of a packed column. Separation takes place when the mobile phase carries the vaporized sample through the column. Different analytes undergo interaction with the stationary phase. When the interaction between the analyte and the stationary phase is stronger, it will take more time to migrate through the column. The time taken for a compound to travel through the column is known as its retention time. Detectors could be mass spectrometry (MS), Flame Ionization Detectors (FID), Photoionization Detectors (PID) and Thermal Conductivity Detectors (TCD).[16] Thin Layer Chromatography (TLC) / High-Performance Thin Layer Chromatography (HPTLC) are simple, cost-effective techniques for qualitative and quantitative analysis. TLC works on the principle of differential partitioning (or adsorption) of compounds between a stationary phase and a mobile phase. The stationary phase is a thin layer of adsorbent material (most commonly silica gel, alumina, or cellulose or a non-polar sorbent) coated onto an inert support plate (glass, plastic, or aluminum). The mobile phase is a liquid solvent or a mixture of solvents that moves up the stationary phase by capillary action. The mobile phase can be of varying polarity, which differs according to the analyte's nature.[17,18] High-Performance Liquid Chromatography (HPLC) is widely used for separating and quantifying active pharmaceutical ingredients, impurities, and degradation products in various matrices. HPLC involves a column (stationary phase) through which a sample is passed under pressure. Each

component of the sample mixture will interact differently with the stationary phase and using different compositions of mobile phase and different flow rates, the sample components will be eluted, resulting in the proper separation. HPLC is incredibly versatile, especially when combined with different detectors, such as UV-Visible spectroscopy (UV-Vis), mass spectrometry (MS), and fluorescence.[19,20] Electrochemical Methods: These techniques involve measuring electrical properties, such as current, voltage, and charge, to detect and quantify chemical species. For example, potentiometry is the measurement of an electrochemical cell's potential. Ion-selective electrodes are frequently used in potentiometric assays to estimate the concentration of specific ions, which is crucial for pharmaceutical formulations.[21] Voltammetry is a technique for current measurement under an applied voltage. It is well known for its sensitivity and capacity to provide extensive information on the electrochemical behavior of the analytes. This category includes techniques such as differential pulse voltammetry, cyclic voltammetry, and square wave voltammetry, which analyze the electrochemical properties of compounds by measuring current changes.[21] Different analytical methods for the quantification of CIT and PIR, either in their pharmaceutical formulations or in biological fluids, will be discussed in this review, focusing on the period from 2012 to 2025. It is important to mention that the official method of analysis for PIR is a chromatographic method described in the British[7] and European pharmacopeia[22] for the determination of PIR in its pure form and pharmaceutical formulations. CIT has an official monograph in the United States Pharmacopoeia (USP)[10], which describes a chromatographic method for its determination.

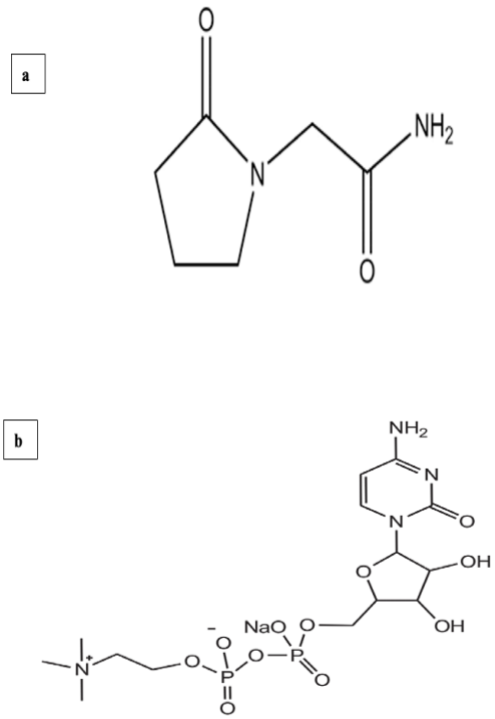

Figure 1. Chemical structure of (a) Piracetam and (b) Citicoline sodium

2. Reported methods of analysis of PIR

2.1. UV Spectrophotometric methods

PIR was analyzed both in its pure form and in its pharmaceutical formulation using a first-order derivative spectrophotometric technique, with peak intensity recorded at 214.0 nm.[23]

Chemometric techniques such as Partial Least Squares (PLS) and Principal Component Regression (PCR), which utilize spectral data, were employed for the quantification of PIR and Cinnarizine in tablet formulations.[24]

The simultaneous estimation of PIR and Nicergoline in their combined dosage form was carried out using zero-order spectrophotometry at 205.0 nm and 287.0 nm. The method demonstrated linearity over the concentration range of 5.00–40.00 µg/mL for both compounds.[25]

2.2. Chromatographic methods

2.2.1. Gas chromatography

Two gas chromatographic techniques have been utilized for the analysis of PIR. The first gas chromatographic method was reported for the determination of PIR in human plasma using fused silica capillary column (8m×0.25mm I.D.) as a stationary phase, where helium was used as carrier gas and the detector used was Nitrogen-Phosphorus flame-ionization detector. The method was linear covering the concentration range of 1.00–30.00 µg/mL.[26]

Another gas chromatographic method was reported for determination of PIR in human plasma using Fused silica capillary column (25m×0.25mm X 0.05 µm) as a stationary phase, nitrogen as a carrier gas and the detection was performed by Nitrogen-Phosphorus flame-ionization detector. The method showed good linearity over the concentration range of 0.10 to 100.00 pg/0.5 mL plasma.[27]

2.2.2. TLC methods

A TLC-densitometric method was developed for the determination of PIR in the presence of its related impurities. The separation was performed on silica gel plates using a mobile phase composed of pentyl acetate: ethyl acetate: ethanol: glacial acetic acid (10:10:9:1 by volume). Spot detection was carried out using UV light at sample and reference wavelengths of 210.0 and 230.0 nm, respectively.[28]

Another TLC-densitometric approach was established for the analysis of PIR and Vincamine in the presence of their degradation products. The separation was carried out on silica gel plates using a mobile phase consisting of chloroform: methanol:

glacial acetic acid: triethylamine (8:2:0.1:0.1 by volume). Spots were detected under UV light at 230.0 nm.[29]

2.2.3. HPLC methods

Multiple HPLC analytical methods have been utilized for the determination of PIR, and a summary of these approaches is provided in **Table 1**.

2.2.4. Capillary electrophoresis methods

PIR was quantified in spiked human plasma using a capillary electrophoresis technique, demonstrating linearity over the concentration range of 4.00–24.00 µg/mL. The experiment was performed using unmodified silica capillary 58 cm (separation distance 51 cm) x 50µm I.D, using a buffer solution of 10 mM borax (pH 9.36) with the addition 40 mM of α-cyclodextrin. The applied voltage was 25 kV.[36] Additionally, a micellar electrokinetic chromatographic method was developed for the analysis of PIR in plasma and cerebrospinal fluid, showing a linear response within the concentration range of 5.00–500.00 µg/mL. The experiment was performed using uncoated fused-silica capillary of 40.2cm, (effective length 30cm) ×50µm I.D, using 60mM Trisbuffer. The applied voltage was 11kV.[37]

2.2.5. Electrochemical Methods

The voltammetric behavior of PIR and Fenotropil was investigated using a solid contact electrode. The developed method was successfully applied for the determination of both drugs in bulk substances, pharmaceutical dosage forms, and human plasma. [38]

A novel molecularly imprinted polymeric sensor was developed for the detection of PIR, utilizing a pencil graphite electrode. The sensor was fabricated through the anodic electropolymerization of o-phenylenediamine (o-PD) in the presence of PIR as the template molecule, which was subsequently removed to activate the sensor. As PIR is electrochemically inactive, the sensor employed $[Fe(CN)_6]^{3-/4-}$ as a redox probe to produce voltammetric signals. The sensor showed linearity in the concentration range of 1.00 $x10^{-13}$- 1.00 $x10^{-12}$ M. The sensor exhibited excellent accuracy and selectivity for PIR in pure solutions, pharmaceutical formulations, and human plasma, achieving a detection limit as low as 4.38×10^{-15} M.[39]

Table 1. HPLC methods for determination of Piracetam.

Column	Mobile phase	Flow rate/runtime	Detector	Application
Nucleosil C18 (25cm x 0.46cm, 10 µm)	Triethylamine 1.0 g/L) : Acetonitrile (70:30 v/v)	1.1 mL/ min run time : 17 mins	UV detection at 205.0 nm.	Simultaneous determination of PIR and Levetiracetam in pharmaceutical and biological samples.[30]
C_{18} (250mm x 4.0 mm, 5µm).	Acetonitrile: Potassium dibasic phosphate 1g /L, (10:90 v/v)	1.0 mL/ min run time: 24 mins	UV detection at 205.0 nm.	Comparative analysis of PIR using HPLC-DAD, HPLC-ESI-MS, and DIP-APCI-MS techniques.[31]
C_{18} (250mm x 4.0 mm ,5µm).	Acetonitrile: Ammonium formate of pH=6 (10:90 v/v)	400.0 µL/ min run time: 24 mins.	Mass spectrometry.	Comparative analysis of PIR using HPLC-DAD, HPLC-ESI-MS, and DIP-APCI-MS techniques.[31]
C_8 column.	0.05 M KH2PO4 solution containing 0.1% triethylamine adjusted at pH 3.0 : Methanol (95:5 v/v)	1.0 mL/min. run time: 10 mins.	UV detection at 230.0 nm.	Analysis of PIR and Vincamine in the presence of their degradation products.[29]
Hypersil gold C_{18} (100 x 4.6 mm, 10µm).	Acetonitrile: Water at pH 2.7 (50:50 v/v)	0.5 mL/min. run time: 15 mins.	UV detection at 229.0 nm.	Simultaneous quantification of Cinnarizine and PIR in capsule formulations.[32]
Promosil C_{18} (100 mm x 4.6 mm , 5µm).	Acetonitrile: Water containing 0.1% TEA of pH 6.5 (30:70 v/v)	0.6 mL/min. run time: 12 mins.	UV detection at 215.0 nm.	Simultaneous determination of Brivaracetam, PIR, and Carbamazepine in formulations and plasma.[33]
Zorbax Eclipse plus C18 (250 mm x 4.6 mm, 5µm)	Methanol and water in gradient elution mode.	1.0 mL/min run time: 8 mins.	UV detection at 270.0 nm.	Quantification of PIR, Ketoprofen, and Omeprazole across various formulation.[34]
Kromasil C18 (250 mm ×4.6 mm, 5 µm)	Potassium dihydrogen phosphate buffer (0.05 M) (pH 3.5): Ethanol absolute (60:40 v/v).	1.0 mL/min.\ run time: 5mins	UV detection at 220.0 nm.	Assay of PIR and Vincamine in pharmaceutical products.[35]

3. Methods of analysis of CIT

3.1. Spectroscopic methods

CIT was quantified using a zero-order spectrophotometric method at 272.0 nm, exhibiting linearity across the concentration range of 5.00–50.00 µg/mL.[40]

A second derivative spectrophotometric technique was applied for analyzing CIT in pharmaceutical formulations, where the peak intensity was recorded at 274.60 nm. Additionally, an absorbance correction approach was employed for the simultaneous detection of PIR and CIT in their combined dosage form at 206.8 nm.[41]

The ratio-derivative spectrophotometric method was utilized for the simultaneous estimation of Edaravone and CIT in laboratory-prepared mixtures. This approach showed linear responses within 1.00–6.00 µg/mL for Edaravone and 25.00–150.00 µg/mL for CIT, with absorbance readings taken at 267.0 nm and 258.4 nm for Edaravone and CIT, respectively.[42]

CIT was analyzed using a zero-order spectrophotometric method at 270.0 nm, demonstrating linearity over the concentration range of 10.00–60.00 µg/mL. This method was successfully applied to assess CIT in its pharmaceutical dosage form.[43]

Another spectrophotometric technique was described for the determination of CIT in syrup formulations, where zero-order measurements were taken at 280.0 nm. The method exhibited linearity within the concentration range of 16.00–24.00 µg/mL.[44]

3.2. Spectroflourimetric methods

A sensitive spectroflourimetric method was developed for the quantification of CIT in pharmaceutical formulations. The method is based on the formation of a binary complex between CIT and Eosin Y in an acidic environment using acetate buffer at pH 3.6, which results in a measurable quenching of Eosin Y's native fluorescence. The decrease in fluorescence intensity was recorded at an emission wavelength of 540.0 nm following excitation at 518.0 nm.[45]

3.3. Chromatographic methods

3.3.1. Gas chromatographic methods:

A gas chromatography technique was described for analyzing organic volatile impurities in CIT The method employed a Supelcowax 25301-U capillary column (30 m × 0.53 mm × 1.0 µm) as the stationary phase, with nitrogen used as the carrier gas. Detection of the analytes was carried out using a Flame Ionization Detector (FID).[46]

3.3.2. TLC method

A TLC-densitometric technique was established for the determination of CIT

alongside its related impurities. The separation was carried out on silica gel plates using a mobile phase consisting of ammonia, ethyl acetate, and triethylamine in a volumetric ratio of 6:3.5:0.5 (by volume). Detection of the spots was performed under UV light at 254.0 nm.[47]

3.3.3. HPLC methods

Various HPLC techniques have been employed for the quantification of CIT, and a summary of these methods is presented in **Table 2.**

3.4. Electrochemical Methods

Two liquid-contact potentiometric ion-selective electrodes were developed for the quantification of CIT in pharmaceutical dosage forms. The first sensor exhibited a linear response over the concentration range of 6.30×10^{-6} to 1.00×10^{-3} M with a slope of 55.9 mV, while the second sensor showed linearity from 1.00×10^{-5} to 1.00×10^{-3} M with a slope of 51.8 mV.[53]

Three solid-contact potentiometric ion-selective electrodes were developed for the determination of CIT in bulk substance, pharmaceutical formulations, and spiked human plasma. This study marks the first use of cobalt oxide and copper-based nanocomposites as ion-to-electron transducer layers in solid-state sensors for the potentiometric measurement of CIT in any biological fluid. The proposed electrodes showed linearity in the concentration range of 1.00×10^{-4} to 1.00×10^{-2} M, and 1.00×10^{-8} to 1.00×10^{-2} M, in the case of the bare and the modified glassy carbon electrodes, respectively.[54]

4. Methods for simultaneous analysis of PIR and CIT

4.1. UV Spectrophotometric methods

An absorbance correction technique was employed to simultaneously estimate PIR and CIT in their combined formulation at 206.8 nm, however, CIT was quantified solely in its pharmaceutical preparations using a second derivative spectrophotometric method, where its peak intensity was observed at 274.6 nm.[41]

Both the absorbance correction and Q-Absorbance methods were applied for the simultaneous quantification of CIT and PIR in their combined dosage form. In the absorbance correction approach, CIT was measured at 266.0 nm, where PIR exhibited zero absorbance. PIR's absorbance was then determined by deducting CIT's contribution, and its resulting spectrum was evaluated at 266.5 nm. The Q-Absorbance method utilized Q-analysis calculations at two wavelengths, 220.0 nm (the maximum absorption of PIR) and 228.0 nm (the iso-absorptive point).[55]

Table 2. Summary of HPLC Techniques Used for the Determination of Citicoline Sodium.

Column	Mobile phase	Flow rate/ Run time	Detector	Application
Phenomenex C18 (250 mm x 4.6 mm, 5 µm)	Acetonitrile : Phosphate buffer at pH 5.0 (55: 45 v/v).	1.0 mL/min run time: 15 mins.	UV detection at 270.0 nm.	Analysis of CIT in its pharmaceutical dosage form.[48]
Phenomenex Luna C18 (250 mm x 4.6 mm, 5 µm)	Acetonitrile : Phosphate buffer at pH 5.0 (60: 40 v/v).	1.0 mL/min. run time:6 mins	Detection was carried at 554.0 nm	Simultaneous determination of CIT and Methylcobalamin in pharmaceutical preparations.[49]
C8 (250 mm x 4.6 mm, 5 µm)	Phosphate buffer : Methanol (70:30 v/v).	1.5 mL/min. run time:20 mins	UV detection at 294.0 nm.	Estimation of CIT and methyl paraben in liquid oral formulations.[50]
Zorbax SB-C18 (150 mm x 4.6 mm, 5 µm)	Methanol: Water: Acetic acid at pH 4.0 (60:40:0.1 by volume).	1.0 mL/min. run time:5 mins	UV detection at 272.0 nm.	Determination of CIT in the presence of its alkaline degradation products.[47]
Atlantis HILIC Si column (50 mm x 4.6 mm, 3 µm)	Acetonitrile : 0.02 M Formate buffer at pH 3.0 (70: 30 v/v).	1.0 mL/min. run time: 15 mins	UV detection at 270.0 nm.	Detection of CIT in the presence of degradation products.[51]
Eurosphar Column (150 mm x4.6 mm, 5µm)	Water: Methanol : Acetonitrile (20:20:60 by volume).	1.0 mL/min. run time: 11 mins	UV detection at 247.0 nm.	Simultaneous determination of CIT and Simvastatin, in their combined dosage form.[52]

The simultaneous equation method was applied for the concurrent estimation of CIT and PIR in their combined tablet dosage forms, utilizing 280.3 nm and 264.1 nm as the selected wavelengths for CIT and PIR, respectively. Additionally, an absorbance ratio method was described for the analysis of both drugs, using 256.6 nm (the iso-absorptive point) and 280.3 nm (the λ_{max} of CIT) to construct the Q-absorption ratio equation.[56]

An absorption correction technique was also documented for the simultaneous determination of PIR and CIT in their combined dosage form. In this method, PIR was quantified at 220.0 nm by subtracting the absorbance of CIT from the total absorbance.

The method showed linearity within the concentration range of 50.00–150.00 µg/mL and 100.00–300.00 µg/mL for CIT and PIR, respectively.[57]

4.2. Chromatographic methods

4.2.1. TLC methods

A TLC-densitometric method was developed for the simultaneous determination of CIT and PIR in their combined dosage form. Separation was achieved on silica gel plates using a mobile phase consisting of methanol and water in a 16:4 (v/v) ratio. Spot detection was carried out under UV light at 212.0 nm.[58]

An alternative TLC-densitometric method was developed for the simultaneous analysis of CIT and PIR in the presence of their degradation products. Separation was performed on silica gel plates using a mobile phase composed of methanol, chloroform, and ammonium chloride buffer in a 9:1:2 by volume ratio. Detection of the spots was conducted under UV light at 230.0 nm.[59]

4.2.2. HPLC methods

A summary of multiple HPLC analytical techniques that have been used to determine PIR and CIT is given in **Table 3**.

5. Conclusion:

A wide array of analytical techniques has been employed for the determination of PIR and CIT in pharmaceutical formulations and biological samples. Among these, high-performance liquid chromatography (HPLC) has been most extensively utilized, particularly for the analysis of PIR and CIT in different pharmaceutical preparations and biological matrices such as plasma. It is important to note that a short chromatographic run time facilitates the routine analysis of multiple samples which is highly beneficial for pharmaceutical testing in quality control laboratories. The reported methods.[59,63] are considered the most applicable ones for PIR and CIT analysis in quality control labs due to their short run time of approximately 5 mins. This review provides a comprehensive overview of the current state-of-the-art analytical approaches for the quantification of PIR and CIT.

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7. Declaration of Competing Interests

The authors declare that they have no competing interests.

Table 3: Overview of HPLC Methods for Piracetam and Citicoline Sodium Determination.

Column	Mobile phase	Flow rate/ Run time	Detector	Application
Phenomenex Gemini C18 (250mm x 4.0 mm, 5µm).	Phosphate buffer : Acetonitrile (95:5 v/v) adjusted at pH=6.9	1.0 mL/ min. run time :7 mins	UV detection at 220.0 nm.	Determination of PIR and CIT in their combined dosage form.[60]
Chromatopak C_{18} (250mm x 4.0 mm, 5µm).	Phosphate buffer : Methanol (90:10 v/v) adjusted at pH=3.5	0.8 mL/ min run time: 10 mins	UV detection at 210.0 nm.	Determination of PIR and CIT in tablets.[61]
Inertsil C_{18} (250mm x 4.0 mm, 5µm).	Phosphate buffer : Acetonitrile in gradient elution mode	1.0 mL/ min. run time: 45 mins	UV detection at 205.0 nm for PIR and 280.0 nm for CIT.	Determination of PIR and CIT in pharmaceutical dosage form. 62
Phenomenex C_8 (250 mm x 4.6 mm , 5µm)	Water (containing 0.1% TEA): Ethanol (92:8 v/v)	0.6 mL/min run time: 5 mins.	UV detection at 230.0 nm.	Estimation of PIR and CIT in presence of degradation products. 59
Thermo Scientific C_{18} (250 mm x 4.6 mm , 5µm).	Phosphate buffer: Acetonitrile (60:40 v/v).	1.0 mL/min. run time: 5 mins.	UV detection at 265.0 nm.	Analysis of PIR and CIT in their combined formulations. 63

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9. Authors Contribution:

Passant M. Medhat: writing the original draft, **Nermine S. Ghoniem**: Supervision, review & editing, **Manal Mohamed Fouad**: Supervision, review& editing, **Hany H. Monir**: Supervision, review & editing, and **Heba-Alla H. Abd-ElSalam**: Supervision, review& editing. All authors approved the final manuscript.

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