Review Article

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# Insights on Different Analytical Methods for Determination of Anti-Flu Drugs

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**Abstract**

*In this literature review, we will introduce most reported methods that have been developed for determination of certain anti-flu drugs such as caffeine, phenylephrine, paracetamol, and ascorbic acid in their pure form, combined form with other drugs, combined form with degradation products, and in biological samples.*

## 1. Metods for analysis of Caffeine

### 1.1. Chromatographic methods:

#### 1.1.1. HPLC methods:

| Stationary phase | Mobile phase | Detection | Specification | REF |
|---|---|---|---|---|
| Bondapack $C_8$ column | 0.01 M $KH_2PO_4$-methanol-acetonitrile-isopropyl alcohol (420: 20: 30: 30) (v/v/v/v) | 215 nm | HPLC Method for the Analysis of Paracetamol, Caffeine and Dipyrone | [1] |
| $C_{18}$ column | water–methanol–ethyl acetate–phosphoric acid, | 210 nm | Simultaneous determination of catechins, caffeine and other phenolic compounds in tea using new HPLC method | [2] |
| Bio SiL HL $C_{18}$, 5 µm, 250×4.6 mm column. | acetonitrile–water (25:75 v/v) | 207 nm | HPLC assay of acetylsalicylic acid, paracetamol, caffeine and phenobarbital in tablets | [3] |
| reversed phase $C_{18}$ column | 1–85% acetonitrile in water (pH 3.0) | 280 nm | A validated HPLC method for the determination of pyridostigmine bromide, acetaminophen, acetylsalicylic acid and caffeine in rat plasma and urine | [4] |
| $C_{18}$ column | acetonitrile–phosphate buffer 50mM | 210 nm | Simultaneous determination of catechins, caffeine and gallic acids in green, Oolong, black and puerh teas using HPLC with a photodiode array detector | [5] |
| Hypersil CN column (150×5.0 mm, 5 µm) | acetonitrile, an ion-pair solution and tetrahydrofuran (13:14:87, v/v, pH4.5) | 223 nm | Simple HPLC method for simultaneous determination of acetaminophen, caffeine and chlorpheniramine maleate in tablet formulations. | [6] |

| | | | | |
|---|---|---|---|---|
| Zorbax Eclipse XDB-$C_8$ column (4.6 × 150 mm i.d., 5 µm particle size) | water-THF (0.1% THF in water, pH 8)-acetonitrile (90:10, v/v) | 273 nm | Simultaneous HPLC Determination of Caffeine, Theobromine, and Theophylline in Food, Drinks, and Herbal Products | [7] |
| A 25 cm × 4.6 mm id LiChrosorb RP-18 5 µm column | The solvents compositions used were (A) water–acetonitrile–formic acid (94.7:4.3:1 v/v) and (B) water–acetonitrile–formic acid (49.5:49.5:1 v/v). The mobile phase composition started at 90% solvent A and 10% solvent B, | 275 nm | HPLC determination of catechins and caffeine in tea. Differentiation of green, black and instant teas | [8] |
| Atlantis $C_{18}$ column | 15 mM potassium phosphate (pH 3.5) and acetonitrile (83:17, v/v) | 274 nm | Validated HPLC Method for Determination of Caffeine Level in Human Plasma using Synthetic Plasma: Application to Bioavailability Studies* | [9] |
| Onyx Monolithic $C_{18}$ (100 × 4.6 mm) | acetonitrile and phosphate buffer (pH 6.50) (90, v/v) | 210 nm | Development and validation of a rapid HPLC method for the determination of ascorbic acid, phenylephrine, paracetamol and caffeine using a monolithic column. | [10] |
| C18 reversed-phase column | methanol/water/ortho phosphoric acid | 230 nm | Isocratic elution system for the determination of catechins, caffeine and gallic acid in green tea using HPLC | [11] |
| phenyl column (2.1 × 150 mm) | acetonitrile, glacial acetic acid and deionized water (8:1:91 v/v/v) | 280 nm | Simple isocratic method for simultaneous determination of caffeine and catechins in tea products by HPLC | [12] |
| Kinetex-$C_{18}$ (4.6 mm, 150 mm, 5 mm) column | 10 mM phosphate buffer (pH 3.3) and acetonitrile | 230 nm | Development and validation of a novel RP-HPLC method for simultaneous determination of paracetamol, phenylephrine hydrochloride, caffeine, cetirizine and nimesulide in tablet formulation. | [13] |
| column RP-18 | water, acetonitrile, methanol, ethyl acetate, glacial acetic acid (89:6:1:3:1 v/v/v/v/v) | 280 nm | A method for fast determination of epigallocatechin gallate (EGCG), epicatechin (EC), catechin (C) and caffeine (CAF) in green tea using HPLC | [14] |
| $C_{18}$ column | 0.05% acetic acid / methyl alcohol (92.5:7.5, v/v) | 280 nm | Liquid chromatographic method for the simultaneous determination of caffeine and fourteen caffeine metabolites in urine | [15] |

### 1.1.2. Thin Layer Chromatographic methods:

| Stationary phase | Mobile phase | Detection | Specification | REF |
|---|---|---|---|---|
| Silica gel 60F254 aluminum plate | acetone-methanol | 274 nm | Quantitative thin-layer chromatographic analysis of ergotamine tartrate and caffeine in the nanogram range | [16] |
| Silica gel aluminum plates 60 F-254 | Chloroform: Acetone (8.8:1.2) | 274 nm | Validated High-Performance Thin Layer Chromatographic Method for Caffeine Quantification in Beverages and Edibles | [17] |
| silica gel G | 1.0 N aqueous sodium hydroxide solution, methanol and chloroform (10:40:50) | spraying with chloramine-T spray reagent I | A Thin-Layer Chromatographic Procedure for the Separation of Aspirin, Cocaine, Caffeine, Codeine, Heroin, 6-Monoacetyl Morphine, and Morphine | [18] |
| Aluminum Plates Of Silica gel 60 F254 | ethyl acetate:methanol in the proportion of 27: 3 (v/v) | 274 nm | Study of extraction and HPTLC - UV method for estimation of caffeine in marketed tea (Camellia sinensis) granules | [19] |
| RP-HPTLC-W18 chromatographic plates | methanol:glacial acetic acid:water (25:4.3:70.7; v:v:v) | 254 nm | Quantitative Evaluation of Paracetamol and Caffeine from Pharmaceutical Preparations Using Image Analysis and RP-TLC | [20] |
| LiChrospher silica gel plates | acetone/toluene/chloroform (4:3:3, v/v/v) | 274 nm | Determination of caffeine, theobromine and theophylline in Mate beer and Mate soft drinks by high-performance thin-layer chromatography | [21] |
| silica HPTLC plates | microemulsion (SDS: water: n-hexane: 1-butanol, 8 g: 8 ml: 160 ml: 25 ml) | 272 nm | Microemulsion Thin-layer Chromatographic Separation of Caffeine and Paracetamol and their Determination in Formulated Tablet and in Spiked Urine Sample by HPLC | [22] |

### 1.2. Spectrophotometric methods:

| Method of determination | Detection | Specification | REF |
|---|---|---|---|
| Simultaneous equation | 216 and 300 nm | PLS-UV spectrophotometric method for the simultaneous determination of paracetamol, acetylsalicylic acid and caffeine in pharmaceutical formulations | [23] |
| Simple and derivative spectrophotometry | 200-350 nm | Simultaneous spectrophotometric determination of phenilpropanolamine HCL, caffeine and diazepam in tablets | [24] |
| Inverse least square Principal component analysis | 225–285 nm | Spectrophotometric multicomponent analysis of a mixture of metamizol, acetaminophen and caffeine in pharmaceutical formulations by two chemometric techniques. | [25] |
| Derivative ratio spectra-zero crossing procedure | 244.8–276.9 nm | Derivative ratio spectra-zero crossing spectrophotometry and LC method applied to the quantitative determination of paracetamol, propyphenazone and caffeine in ternary mixtures | [26] |

| | | | |
|---|---|---|---|
| Ratio spectra spectrophotometry and chemometric methods viz, classical least squares, and inverse least squares | 225–285 nm | Simultaneous spectrophotometric determination of chlorphenoxamine hydrochloride and caffeine in a pharmaceutical preparation using first derivative of the ratio spectra and chemometric methods. | [27] |
| Multivariate calibration and N-way partial least squares (PLS) | 210–300 nm | N-way PLS applied to simultaneous spectrophotometric determination of acetylsalicylic acid, paracetamol and caffeine | [28] |
| Multivariate calibration method and chemometric methods viz, partial least squares, and principle component regression | 190–300 nm | Simultaneous determination of phenytoin, barbital and caffeine in pharmaceuticals by absorption (zero-order) UV spectra and first-order derivative spectra-multivariate calibration methods | [29] |
| Continuous wavelet transform and derivative transform (using Savitzky-Golay filters) | 220–300 nm | Simultaneous spectrophotometric determination of overlapping spectra of paracetamol and caffeine in laboratory prepared mixtures and pharmaceutical preparations using continuous wavelet and derivative transform | [30] |
| H-point standard addition method | 453 nm | Simultaneous kinetic determination of paracetamol and caffeine Cu(II)-neocuproine in presence of dodecyl sulfate by H-point standard addition method | [31] |
| Simultaneous equation method and Q-absorbance equation at isosbestic point | 200–400 nm | Simultaneous spectrophotometric determination of paracetamol and caffeine in tablet formulation | [32] |
| Isoabsorption assay method | 200–300 nm | Estimation of caffeine and sodiun benzoate in caffeine and sodium benzoate injection by isoabsorption method (isobestic method) | [33] |
| Simultaneous equation method and absorbance ratio method | 200–400 nm | Development of validated spectrophotometric method for simultaneous estimation of acetylsalicylic acid and caffeine in pure and tablet dosage form. | [34] |

### 1.3. Spectrofluorimetric methods:

PLS-1, a variant of the partial least-squares algorithm was used for the solid-phase spectrofluorimetric determination of acetylsalicylic acid (ASA) and caffeine (CAF) in pharmaceutical formulations. The method allows the simultaneous quantification of the analytes, as the closely overlapping spectral bands are efficiently solved. Sample preparation prior to analysis is not required. The alignment set comprised of 83 examples with 50–170 mg g−1 ASA in addition to 5–20 mg g−1 CF; another arrangement of 25 examples was utilized for outer approval. Understanding among anticipated and test focuses was reasonable (r = 0.987 and 0.974 for ASA and CAF models). For the two models, the forecast exhibition was assessed as far as the coefficient of fluctuation (CV), relative prescient assurance (RPD), and proportion mistake extend (RER). The last PLS-1 models were utilized for the assurance of ASA and CAF in pharmaceutical plans [35].

Partial least-squares algorithm (PLS) -1 was used for the solid-phase spectrofluorimetric determination of paracetamol (PAR) and caffeine (CAF) in pharmaceutical formulations. In spite of the intently covering phantom groups, the technique permits the concurrent measurement and test arrangement before investigation isn't required. The alignment set comprised of 96 examples with 100–400 mg/g−1 PA in addition to 10–65 mg/g−1 CF; another arrangement of 25 examples was utilized for outside approval. Understanding among anticipated and exploratory focuses was reasonable (r=0.993 and 0.964 for PAR and CAF models). Forecast execution was assessed regarding the coefficient of changeability (CV), relative prescient assurance (RPD), and proportion mistake go (RER). The PLS-1 model was utilized for the assurance of PA and CF in pharmaceutical plans [36].

### 1.4. Capillary electrophoresis methods:

The determination of caffeine and its analogues is important for a wide variety of analyses and is performed in an assortment of matrices ranging from food to clinical samples. While reversed-phase HPLC has become the standard analysis protocol in most laboratories, capillary electrophoresis has the advantages of higher separation efficiency and shorter separation time. The micellar hairlike electrophoresis (MECC) detachment of caffeine and its metabolites, theobromine, paraxanthine, theophylline and 1, 3, 7-trimethyluric corrosive was researched utilizing sodium dodecyl sulfate (SDS) as the micellar stage. The impacts of pH, micelle focus, cradle fixation, ionic quality, cushion salts, applied voltage and infusion time were concentrated to choose the ideal conditions for the assurance of caffeine and its four analogs in medications, nourishments and body liquids. Caffeine and its three analogs were settled inside 120 s with recognition restrains under 1 µg/ml. Samples could be analyzed utilizing direct injection with satisfactory resolution and reproducibility [37].

Capillary zone electrophoresis (CE) and micellar electrokinetic capillary chromatography (MEKC), with detection at 200 and 220 nm, were investigated for analytes in human serum and urine. When adequae partition was not accomplished in starter concentrates with CE, further advancement was centered around the MEKC technique. Glycine support containing sodium lauryl

sulfate (pH 10.5) was used for the MEKC partitions. The analytes and carboxylic acids utilized as marker mixes could be screened by a short-slender strategy in lessthan 2 min. In the synchronous assurance of the medications in pee and serum and longer partition of 18 min was liked with the goal that all the aggravates, the markers and the endogenous mixes retaining at the discovery wavelenght could be satisfactorily isolated in a solitary run. The movement seasons of the mixes expanded in the request caffeine, morphine, heroin, codeine and amphetamine. The repeatability of the partition was tried by utilizing two carboxylic acids as marker mixes in the assurance of the relocation files of the analytes. The relative standard deviations for the relocation lists were under 1%, which is exact enough for the assurance of the medications in natural liquids [38].

## 2. Metods for analysis of Phenylephrine

### 2.1. Chromatographic methods:

#### 2.1.1. HPLC methods:

| Stationary phase | Mobile phase | Detection | Specification | REF |
|---|---|---|---|---|
| Thermo BDS Hypersil $C_{18}$ column (250 mm Ã— 4.6 mm, 5 µm) | Methanol: Phosphate buffer (30:70v/v) | 215 nm | RP-HPLC method development and validation for simultaneous estimation of phenylephrine hydrochloride and ebastine in tablet dosage form | [39] |
| silica column | methanol: water (containing 6.0 g of ammonium acetate and 10 ml of triethylamine per liter, pH adjusted to 5.0 with orthophosphoric acid), 95:5%(v/v) | 254 nm | A New HPLC Method for the Simultaneous Determination of Acetaminophen, Phenylephrine, Dextromethorphan and Chlorpheniramine in Pharmaceutical Formulations. | [40] |
| $C_{18}$ column | Solvent A: phosphate buffer 40 mM at pH 6.0 and solvent B: acetonitrile. At t=0, the mobile phase consisted of 92% A and 8% B | 215 & 280 nm | Validation of a HPLC quantification of acetaminophen, phenylephrine and chlorpheniramine in pharmaceutical formulations: capsules and sachets | [41] |
| reversed-phase $C_{18}$ column (250 mm × 8 mm i.d., particle size 10 µm) column | acetonitrile and phosphate buffer 55:45 (v/v) | 255 nm | Development and validation of a RP-HPLC method for the determination of chlorpheniramine maleate and phenylephrine in pharmaceutical dosage form | [42] |
| HS PEG column (polyethylene glycol), 5 µm | 20 mM phosphate buffer at pH 7.0/acetonitrile 80:20 (v/v) | 210 nm | CE versus HPLC for the dissolution test in a pharmaceutical formulation containing acetaminophen, phenylephrine and chlorpheniramine. | [43] |
| Sunfire $C_{18}$ column (5 µm × 250 mm × 4.6 mm) | acetonitrile : mathanol : phosphate buffer (50 : 20 : 30, v/v/v, pH 5.6) | 220 nm | Development and Validation of an RP-HPLC Method for Estimation of Chlorpheniramine Maleate, Ibuprofen, and Phenylephrine Hydrochloride in Combined Pharmaceutical Dosage Form | [44] |
| XTerra $RP_{18}$ column, 3 µm particle size, 50 × 3.0 mm id. | Acetonitrile and buffer (10 mM sodium octane-1-sulfonate, adjusted with H3PO4 to pH 2.2; 200 + 800, v/v), | 275-310 nm | Fast HPLC Method Using Ion-Pair and Hydrophilic Interaction Liquid Chromatography for Determination of Phenylephrine in Pharmaceutical Formulations | [45] |
| Supelco Discovery HS PEG column poly (ethyleneglycol) 15×0.46 cm, 5 µm. | 20 mM phosphate buffer, pH 7.0–acetonitrile (90:10, v/v) | 215 nm | Poly(ethyleneglycol) column for the determination of acetaminophen, phenylephrine and chlorpheniramine in pharmaceutical formulations | [46] |

| | | | | |
|---|---|---|---|---|
| Agilent Zorbax SB-CN column | 0.02 M phosphate buffer (pH:4) and acetonitrile (85:15,v/v) | 230 nm | Simultaneous Determination of Paracetamol, Phenylephrine Hydrochloride, Oxolamine Citrate and Chlorpheniramine Maleate by HPLC in Pharmaceutical Dosage Forms | [47] |
| reversed-phase column | methanol:water:acetonitrile (80:12:8 v/v/v/) | 270 nm | Determination of phenylephrine hydrochloride and chlorpheniramine maleate in binary mixture using chemometric-assisted spectrophotometric and high-performance liquid chromatographic-UV methods | [48] |

### 2.1.2. Thin Layer Chromatographic methods:

| Stationary phase | Mobile phase | Detection | Specification | REF |
|---|---|---|---|---|
| Silica gel 60F254 aluminum plate | chloroform–methanol–ammonia (7:3:0.1, v/v) and (7.5:2.5:0.1, v/v) | 273 and 320 nm | Development and Validation of a High-Performance Thin-Layer Chromatographic Method for the Simultaneous Determination of Two Binary Mixtures Containing Ketorolac Tromethamine with Phenylephrine Hydrochloride and with Febuxostat | [49] |
| Silica gel aluminum plates 60 F-254 | ethyl acetate–methanol–ammonia (4:1:0.4 v/v/v) | 262 nm and 291 nm | Simultaneous determination of lignocaine hydrochloride and phenylephrine hydrochloride by HPTLC | [50] |
| silica gel | N-butanol-methanol-toluene-water and acetic acid. | spraying with chloramine-T spray reagent I | Simultaneous Quantitative Determination of Codeine Phosphate, Chlorpheniramine Maleate Phenylephrine Hydrochloride and Acetaminophen in Pharmaceutical Dosage Forms Using Thin Layer Chromatography Densitometry | [51] |
| Aluminum Plates Of Silica gel 60 F254 | n-Butanol: Ethanol: Ammonia (6:3.5:0.6, v/v/v) | 288 nm | A Simple and Sensitive HPTLC Method for Simultaneous Analysis of Phenylephrine hydrochloride and Ketorolac tromethamine in Combined Dose Formulation | [52] |
| silica gel | methylene chloride: methanol: glacial acetic acid: ammonia (17.8: 1.68: 0.4: 0.12, v/v) | 257 nm | Robust Chromatographic Methods for the Analysis of Two Quaternary Mixtures Containing Paracetamol, Codeine, Guaifenesin and Pseudoephedrine or Phenylephrine in their Dosage Forms | [53] |
| Silica gel 60 F254 | toluene: acetone: isopropyl alc.: ammonia (5.3: 2.7: 1.8: 0.4, by volume) | 254 nm | 'Purity Indicating TLC Method for Quantitative Determination of Phenylephrine and Dimethindine Maleate in Presence of Dimethindine Maleate Impurity: 2-ethyl pyridine in Nasal Gel. | [54] |

### 2.2. Spectrophotometric methods:

| Method of determination | Detection | Specification | REF |
|---|---|---|---|
| Absorbance ratio methods/ zero-crossing first derivative UV spectrophotometric | 271.6 / 250.2 nm 246.5 / 238.6 nm | Quantitative analysis of chlorpheniramine maleate and phenylephrine hydrochloride in nasal drops by differential-derivative spectrophotometric, zero-crossing first derivative UV spectrophotometric and absorbance ratio methods. | [55] |
| derivative spectrophotometry | 269 nm | Simultaneous high performance liquid chromatographic and derivative ratio spectra spectrophotometry determination of chlorpheniramine maleate and phenylephrine hydrochloride | [56] |
| based on the coupling of 4-aminoantipyrine (4-AAP) with phenylephrine hydrochloride (PEH) to give a new ligand that reacts with copper (II) | 240 nm | Spectrophotometric Assay of Phenylephrine Hydrochloride Using 4-Aminoantipyrine and Copper (II) | [57] |
| Simultaneous equation | 200 to 400 nm | Simultaneous spectrophotometric determination of paracetamol, phenylephrine and chlropheniramine in pharmaceuticals using chemometric approaches | [58] |
| Zero-crossing derivative spectrophotometry | 286.5, 220, 265.8, 262.2, 269.5, and 273.8 nm | Simultaneous spectrophotometric determination of chlorphenoxamine hydrochloride and caffeine in a pharmaceutical preparation using first derivative of the ratio spectra and chemometric methods. | [59] |

### 2.3. Spectrofluorimetric methods:

Two free spectrofluorometric techniques were created for the assurance of phenylephrine (PHE) in pharmaceutical tablets containing an enormous abundance of paracetamol (PAR) (somewhere in the range of 30:1 and 100:1 in weight). One of them includes standard expansion of phenylephrine (in the range 0–2.00 mg l−1 in overabundance regarding the substance of the obscure example) to a marginally corrosive watery arrangement (HCl) of the tablet parts. The constraints of assurance and evaluation were seen as 0.08 and 0.27 mg l−1, separately. A surface reaction improvement approach, utilizing a blocked 3D square star configuration was completed to enhance the factors which have an effect on the fluorescence discharge of the analyte. The ideal conditions acquired were excitation frequency, 277 nm and grouping of paracetamol, 72 mg l−1. As an elective strategy, the multivariate fractional least-squares (PLS) alignment of fluorescence excitation spectra was completed. For this situation the scope of use is 0.80–2.00 mg l−1 of phenylephrine. The two procedures were acceptably applied to a few pharmaceutical tablets. The outcomes gave by the two strategies are not. Statistically different [60].

Simple and rapid synchronous fluorometric methods were adopted and validated for the simultaneous analysis of a binary mixture of diphenhydramine (DIP) and ibuprofen (IBU) (Mix I) or DIP and phenylephrine (PHE) (Mix II) in their co-formulated pharmaceuticals without prior separation. Investigation of Mix I depends on the estimation of the pinnacle amplitudes (D1) of coordinated fluorescence forces at 265.1 nm for DIP and 260 nm for IBU. The connection between the focus and the adequacy of the first-derivative coordinated fluorescence spectra demonstrated great linearity over the fixation ranges 0.50–10.00 µg ml−1 and 0.50–7.90 µg ml−1 for DIP and IBU, separately. Investigation of Mix II depended on estimation of the pinnacle plentifulness (D1) coordinated fluorescence powers at 230 nm for DIP and at 253.9 nm for PHE. Also, for Mix II, the pinnacle abundancy (D2) simultaneous fluorescence forces were estimated at 227.9 nm for DIP and at 264.9 nm for PHE. Alignment plots were rectilinear over the focus run 0.30–3.50 µg ml−1 and 0.03–0.75 µg ml−1 for DIP and PHE, separately. The proposed techniques were effectively applied to decide the considered mixes in unadulterated structure and in pharmaceutical arrangements [61].

### 2.4. Capillary electrophoresis methods:

A capillary zone electrophoresis (CE) method has been developed to separate and quantitate naphazoline (NAPH), dyphenhydramine (DIP) and phenylephrine (PHE) in nasal solutions. Samples were diluted 1:25 in ultrapure water and injected at the anodic end. A focal composite structure has been utilized to improve the test conditions for a total and quick detachment of the dynamic fixings considered. Basic boundaries, for example, voltage, pH and cradle focus have been concentrated to assess how they influence reactions, for example, goal and movement times. Partition was performed on a silica slender with 75 µm I.D. what's more, 70 cm complete length at an applied voltage of 17.7 kV with a phosphate run cradle of pH 3.72 and 0.063 mol l−1. Adjustment bends were set up for NAPH, DIP and PHE. For each analyte, the relationship coefficients were >0.999 (n=15). The RSD% of six duplicate infusions for each analyte were sensibly acceptable. The strategy was applied to the quantitation of the three segments in a business measurement structure. The proposed technique has the benefit of requiring an extremely basic example pretreatment and being quicker than a normal HPLC chromatographic strategy [62].

The separation of basic nitrogenous compounds commonly used as active ingredients in cold medicine formulations by micellar electrokinetic capillary chromatography and capillary zone electrophoresis with direct absorptiometric detection was

investigated. The type and composition of the background electrolyte (BGE) were explored regarding partition selectivity and BGE security. BGE of 10 mM sodium dihydrogenphosphate–sodium tetraborate cushion containing 10 mM SDS and 10% acetonitrile, pH 9.0 was seen as ideal. Dextromethorphan hydrobhromide, diphenhydramine hydrochloride and phenylephrine hydrochloride were gauge isolated in under 11 min, surrendering partition efficiencies of to 494,000 hypothetical plates, reproducibility of rectified pinnacles regions beneath 3% relative standard deviation and focus identification limits from 2.5 to 5.5 µg ml−1. Discovery was performed at 196 and 214 nm [63].

## 3. Metods for analysis of Paracetamol

### 3.1. Chromatographic methods:

#### 3.1.1. HPLC methods:

| Stationary phase | Mobile phase | Detection | Specification | REF |
|---|---|---|---|---|
| Phenomenex $C_8$ column | sodium dihydrogen phosphate buffer (0.05 M): methanol:acetonitrile (85:10:5, v/v/v) | 254nm | HPTLC and RP-HPLC methods for simultaneous determination of Paracetamol and Pamabrom in presence of their potential impurities | [64] |
| dimethylpolysiloxane (Rtx-1) column | phosphate buffer pH 6.3 and acetonitrile (90:10, v/v) | 220 nm | Determination of Paracetamol and Tramadol Hydrochloride in Pharmaceutical Mixture Using HPLC and GC-MS | [65] |
| $C_{18}$ column | methanol-potassium dihydrogen orthophosphate buffer (pH 3; 10 mM) (50:50, v/v) | 220 nm | Chemometric assisted UV spectrophotometric and RP-HPLC methods for simultaneous determination of paracetamol, diphenhydramine, caffeine and phenylephrine in tablet dosage form | [66] |
| $C_{18}$ column | methanol–water (55:45, v/v pH 3 with aqueous formic acid) | 230 nm | Different stability-indicating chromatographic methods for specific determination of paracetamol, dantrolene sodium, their toxic impurities and degradation products | [67] |
| reversed-phase column | methanol-water (60:40, v/v) | 274.0 nm | Simultaneous determination of paracetamol and methocarbamol in tablets by ratio spectra derivative spectrophotometry and LC | [68] |
| reversed-phase HS $C_{18}$ analytical column (250 mm × 4.6 mm i.d., 5 µm particle size) | 50 mmol L−1 sodium dihydrogen phosphate, 5 mmol L−1 heptane sulfonic acid sodium salt, pH 4.2 and (B) acetonitrile | 214 nm | Development and validation of a stability-indicating RP-HPLC method for the determination of paracetamol with dantrolene or/and cetirizine and pseudoephedrine in two pharmaceutical dosage forms | [69] |
| ODS column | 0.05 M $KH_2PO_4$ buffer : acetonitrile (72.5 : 27.5, v/v, pH = 6) | 225 nm | Validated stability indicating RP-HPLC method for determination of paracetamol, methocarbamol and their related substances | [70] |
| microBondapak CN RP analytical column (125 A, 10 microm, 3.9 x 150 mm) | acetonitrile and phosphate buffer (pH 6.22, 78:22) | 220 nm | Simultaneous High-Performance Liquid Chromatographic Determination of Paracetamol, Phenylephrine HCl, and Chlorpheniramine Maleate in Pharmaceutical Dosage Forms | [71] |

#### 3.1.2. Thin Layer Chromatographic methods:

| Stationary phase | Mobile phase | Detection | Specification | REF |
|---|---|---|---|---|
| silica gel G254 | Ethyl acetate: benzene: acetic acid in a ratio (1:1:0.05 v/v/v). | 250 nm | Stability indicating method for the determination of paracetamol in its pharmaceutical preparations by TLC densitometric method | [72] |
| silica gel 60 F254 | chloroform–methanol–glacial acetic acid (9.5:0.5:0.25, by volume) | 225 nm | Stability-Indicating TLC–Densitometric Method for Simultaneous Determination of Paracetamol and Chlorzoxazone and their Toxic Impurities | [73] |

### 3.2. Spectrophotometric methods:

| Method of determination | Detection | Specification | REF |
|---|---|---|---|
| Flow-injection method | 430 nm | Flow-injection spectrophotometric determination of paracetamol in tablets and oral solutions | [74] |

### 3.3. Spectrofluorimetric methods:

A spectrofluorimetrical selective method was designed for determination of paracetamol in tablets. This important technique can be characterized by its sensitivity, simplicity, celerity and cheaper cost than current official methods. The utilized approach includes coumarinic compound arrangement acquired by response among paracetamol and ethylacetoacetate (EAA) within the sight of sulphuric corrosive as impetus. The response item is profoundly fluorescent at 478 nm, being energized at 446 nm. The direct fixation scope of the application was 0.1–0.4 µg/ml of paracetamol and as far as possible was 57 ng/ml. The impact of various factors was examined and upgraded through chemometric procedures. Applying the previously mentioned technique great outcomes were gotten with respect to pharmaceutical details containing paracetamol. Along these lines, it is applicable to propose this gainful strategy for medicament control examination. [75].

It was developed a methodology for spectrofluorimetric determination of Paracetamol (PAR) and Ibuprofen (IBU) in tablets (synthetic mixtures) and biological fluids (urine) coupled with chemometrics. For tablet's determination, it was used PLS (Partial Least Squares) and for urine samples it was used PARAFAC (Parallel Factor Analysis). In both cases, it was possible to quantify both analytes in samples. The proposed methodology showed effectiveness and offered excellent results in the determination of PAR and IBU. Recovery study test was performed in urine samples, and it offered good results also [76].

### 3.4. Capillary electrophoresis methods:

Paracetamol, caffeine and ibuprofen are found in over-the-counter pharmaceutical formulations. One technique depends on high-performance fluid chromatography & diode-array detection and the other on capillary electrophoresis with capacitively coupled contactless conductivity detection. The division by high-performance fluid chromatography with diode-array discovery was accomplished on a C18 section (250×4.6 $mm^2$, 5 µm) with an inclination versatile stage containing 20–100% acetonitrile in 40 mmol $L^{-1}$ phosphate support pH 7.0. The division by capillary electrophoresis with capacitively coupled contactless conductivity location was accomplished on a fused-silica capillary (40 cm length, 50 µm i.d.) utilizing 10 mmol $L^{-1}$ 3,4-dimethoxycinnamate and 10 mmol $L^{-1}$ β-alanine with pH change in accordance with 10.4 with lithium hydroxide as foundation electrolyte. The assurance of each of the three pharmaceuticals was done in 9.6 min by fluid chromatography and in 2.2 min by capillary electrophoresis. Identification limits for caffeine, paracetamol and ibuprofen were 4.4, 0.7, and 3.4 µmol $L^{-1}$ by fluid chromatography and 39, 32, and 49 µmol $L^{-1}$ by capillary electrophoresis, individually. Recovery values for spiked samples were between 92–107% for both proposed strategies [77].

### 3.5. Electrochemical methods:

A straightforward and profoundly specific electrochemical technique was created for the assurance of paracetamol (N-acetyl-p-aminophenol, acetaminophen) and caffeine (3, 7-dihydro-1, 3, 7-trimethyl-1H-purine-2, 6-dione) in fluid media (acetic acid derivation cushion, pH 4.5) on a boron-doped diamond (BDD) electrode utilizing square wave voltammetry (SWV) or differential pulse voltammetry (DPV). Utilizing DPV with the cathodically pre-treated BDD terminal, a division of around 550 mV between the pinnacle oxidation possibilities of paracetamol and caffeine present in twofold blends was gotten. The calibration curves for the concurrent assurance of paracetamol and caffeine indicated an excellent linear response, running from $5.0 \times 10^{-7}$ mol $L^{-1}$ to $8.3 \times 10^{-5}$ mol $L^{-1}$ for the two mixes. As far as possible for the detection limits for the simultaneous determination of paracetamol and caffeine were $4.9 \times 10^{-7}$ mol $L^{-1}$ and $3.5 \times 10^{-8}$ mol $L^{-1}$, separately. The proposed strategy was effectively applied in the concurrent assurance of paracetamol and caffeine in a few pharmaceutical plans (tablets), with results like those got utilizing a superior fluid chromatography technique (at 95% certainty level) [78].

## 4. Metods for analysis of Ascorbic Acid

### 4.1. Chromatographic methods:

#### 4.1.1. HPLC methods:

| Stationary phase | Mobile phase | Detection | Specification | REF |
|---|---|---|---|---|
| Primesep SB column (4.6 250 mm, particle size 5 mm) | 0.1% formic acid in water, 80%; and 0.08% formic acid in acetonitrile, 20%. | 190 and 230 nm | HPLC methods for simultaneous determination of ascorbic and dehydroascorbic acids | [79] |
| single pump/single analytical column HPLC system | phase A (150 mM monochloroacetic acid, 2 mM Na2EDTA, pH 3.0070.05) and 1.5% mobile phase B (100% methanol) from 0 to 1.8 min followed by a step gradient to 20% methanol (1.81–3.2 min) and re-equilibration at 1.5% methanol to 12 min | 515 nm | Development and implementation of an HPLC-ECD method for analysis of vitamin C in plasma using single column and automatic alternating dual column regeneration. | [80] |

| | | | | |
|---|---|---|---|---|
| pre-packed Kromasil 100, C/sub 18/ (5 macro m 25 x 0.46) column | acetonitrile water (60:40; v/v) | 265 nm | Rapid and specific spectrophotometric and RP-HPLC methods for the determination of ascorbic acid in fruits juices and in human plasma | [81] |
| A reverse-phase $C_{18}$ Spherisorb ODS2 (5 lm) stainless steel column (4.6 mm 250 mm) | 0.01% solution of sulphuric acid 10 mM potassium dihydrogen phosphate buffer and acetonitrile in a ratio 60:40 | 245 nm | Comparative study of UV-HPLC methods and reducing agents to determine vitamin C in fruits | [82] |
| Phenomenex Gem-ini C18 250 mm × 4.6 mm i.d. filled with 5 m particles | 50 mM NaHCO3 adjusted with NaOH or phosphoric acid to different pH values, and McIlvaine buffers prepared from 0.2 M Na2 HPO4 and 0.1 M citric acid. | 266 nm | Development of HPLC and UV spectrophotometric methods for the determination of ascorbic acid using hydroxypropyl- -cyclodextrin and triethanolamine as photostabilizing agents | [83] |
| reverse-phase column MAG 1, 250 mm × 4.6 mm, Labiospher PSI 100 $C_{18}$, 5 µm | ethanol and 25 mmol/L sodium dihydrogenphosphate (2.5:97.5, v/v) | 265 and 292 nm | The determination of ascorbic acid and uric acid in human seminal plasma using an HPLC with UV detection | [84] |
| Wakosil II 5C18 RS column | 82.5:17.5 (v/v) 30 mM monobasic potassium phosphate (pH 3.6)–methanol | 250 nm | Simultaneous Determination of Ascorbic Acid and Free Malondialdehyde in Human Serum by HPLC–UV | [85] |
| $C_{18}$ column | methanol–water (55:45, v/v pH 3 with aqueous formic acid) | 230 nm | Spectrophotometric determination of ascorbic acid using copper(II)–neocuproine reagent in beverages and pharmaceuticals | [86] |

### 4.1.2. Thin Layer Chromatographic methods:

| Stationary phase | Mobile phase | Detection | Specification | REF |
|---|---|---|---|---|
| silica gel | water: methanol (95:5 v/v) | 260 nm | Second Derivatives Ultraviolet Spectrophotometry and HPTLC for Simultaneous Determination of Vitamin C, and Dipyrone | [87] |
| silica gel 60 F254 | ethanol: glacial acetic acid: toluene (5.5:1:1.5) | 254 nm | Quantitative estimation of Gallic acid and Ascorbic acid in a marketed herbal medicine: Triphala Churna by High Performance Thin Layer Chromatography | [88] |
| silica gel 60 F254 | 200 nm and 268 nm for acetylsalicylic acid and ascorbic acid. | 257 nm | A Simple and Cost-Effective TLC-Densitometric Method for the Quantitative Determination of Acetylsalicylic Acid and Ascorbic Acid in Combined Effervescent Tablets | [89] |
| silica gel 60 F254 | Ethanol: Acetic Acid (9.5:0.5 v/v) with the Rf value of 0.76–0.03) | 254 nm | Quantitative Estimation of Ascorbic Acid by HPTLC in different varieties of Amla | [90] |
| 10 10 cm; silica gel absorbent STKh-1A and STKh-1VE, 5 – 17m and 8 – 12 m, layer thickness 90 – 120 m and 80 – 100 m | EtOH solution (5%) of phosphomolybdic acid (PMA) and an EtOH solution (0.2%) of 2, 6-DCPIPS. | 254 nm | Method development for quantitative determination of ascorbic acid by high-performance thin-layer chromatography | [91] |

| | | | | |
|---|---|---|---|---|
| silica gel 60 | methanol/water (3:1, v/v) | 380 nm | Simple determination of L-ascorbic acid on TLC by visual detection using autocatalytic reaction | [92] |
| silica gel 60 F254 | acetone:toluene:water:glacial acetic acid (26:4:4:1 v/v) | 269 nm | Stability indicating high-performance thin-layer chromatography method for estimation of ascorbic acid in Hibiscus sabdariffa L. aqueous extract | [93] |
| silica gel 60 F254 | ethyl acetate: acetone: water: formic acid, 10:6:2:2 (%, v/v/v/v) | 254 nm | HPTLC method for simultaneous determination of ascorbic acid and gallic acid biomarker from freeze dry pomegranate juice and herbal formulation | [94] |

### 4.2. Spectrophotometric methods:

| Method of determination | Detection | Specification | REF |
|---|---|---|---|
| Relative standard deviation | 450 nm | Spectrophotometric determination of ascorbic acid using copper(II)–neocuproine reagent in beverages and pharmaceuticals | [86] |
| zero crossing technique | 280 and 272 nm | Second Derivatives Ultraviolet Spectrophotometry And HPTLC for Simultaneous Determination of Vitamin C, and Dipyrone. | [87] |
| Relative standard deviation | 450 nm | Spectrophotometric determination of ascorbic acid by the modified CUPRAC method with extractive separation of flavonoids–La(III) complexes | [95] |
| charge transfer complexation reaction | 580 nm 530 nm | Validated spectrophotometric methods for the determination of amlodipine besylate in drug formulations using 2,3-dichloro 5,6-dicyano 1,4-benzoquinone and ascorbic acid | [96] |
| based on inhibitory effect of ascorbic acid on the Orange G-bromate system | 478 nm | A Highly Sensitive Kinetic Spectrophotometric Method for the Determination of Ascorbic Acid in Pharmaceutical Samples | [97] |
| depending on its ability to reduce the colors of permanganate) and dichromate) | 530nm and 350 nm | Spectrophotometric Determination of Ascorbic Acid in Aqueous Solutions | [98] |
| based on the oxidation of ascorbic acid | 590 nm | Sensitive Spectrophotometric Methods for the Determination of Ascorbic Acid | [99] |
| flow injection analysis (FIA) method | 460 nm | A Spectrophotometric Determination of Ascorbic Acid | [100] |
| based on the oxidation of ascorbic acid | 265 and 262 nm | Spectrophotometric Determination of L-Ascorbic Acid in Pharmaceuticals Based on Its Oxidation by Potassium Peroxymonosulfate and Hydrogen Peroxide | [101] |

### 4.3. Spectrofluorimetric methods:

An effectively performed, quick, dependable and reasonable system for the spectrofluorometric determination of ascorbic corrosive was proposed utilizing acriflavine as a fluorescence quenching reagent. The system depended on the decided quenching impact of ascorbic corrosive on the characteristic fluorescence sign of acriflavine and the response between ascorbic corrosive and acriflavine in Britton–Robinson support arrangement (pH 6) to create an ion-associated complex. The decrease in acriflavine fluorescence intensity was identified at 505 nm, while excitation happened at 265 nm. The connection between quenching fluorescence power (ΔF) and grouping of ascorbic corrosive was straight ($R_2$ = 0.9967) inside the range 2–10 µg/ml and with a discovery breaking point of 0.08 µg/ml. No huge interference was distinguished from different materials regularly found in pharmaceutical healthful tablets. The proposed spectrofluorimetric technique was utilized to decide the measure of ascorbic corrosive in various business pharmaceutical nourishing enhancement tablets with a 95% certainty execution [102].

### 4.4. Capillary electrophoresis methods:

Grapefruit has been known for its accumulation of flavonoids and ascorbic acid. These contents are essential as of their nutritional and antioxidant characters. Hesperidin, naringin, hesperedin and narigenin rutin and ascorbic acid were separated and evaluated in grapefruit juice by capillary electrophoresis with electrochemistry detection (CE-ED). Hesperidin, naringin and

ascorbic acid were present in extract of grapefruit peel with the same technique. The impacts of several CE parameters on the resolution were examined systematically. Under the optimum conditions, the analytes could be well separated within 25 min in a 60 mmol $L^{-1}$ borate buffer (pH 9.0). The response was linear over four orders of magnitude with detection limits (S/N = 3) ranging from $1.4 \times 10^{-7}$ to $1.0 \times 10^{-6}$ g $ml^{-1}$ for the analytes. The method has been successfully determined for the analysis of grapefruit with satisfactory results [103].

Arginine (ARG), ascorbic acid (ASC) and aspartic acid (ASP) are very popular and widely consumed active ingredients used for fatigue treatment or improvement of physical performance. The capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C4D) and with UV spectrophotometric detection (CE-UV) used for determination of ARG, ASC and ASP. The separation by CE-C4D was achieved with a background electrolyte (BGE) composed by 20 mmol $L^{-1}$ N-tris (hydroxymethyl) - methyl]-3-aminopropanesulfonic acid (TAPS) and 10 mmol $L^{-1}$ of NaOH (pH 8.7). The limits of detection (LOD) were 0.01, 0.02 and 0.04 mmol $L^{-1}$ for ARG, ASC and ASP, respectively. The proposed CE-UV method was optimized with a BGE composed by 10 mmol $L^{-1}$ sodium tetraborate (pH 9.4). The limits of detection were 0.03, 0.02 and 0.04 mmol $L^{-1}$ for ARG, ASC and ASP, respectively [104].

Ascorbic acid and zinc are essential nutrients that play important roles in nutrition, immune support, and maintenance of health. The method is depend on capillary electrophoresis with capacitively coupled contactless conductivity detection using a fused silica capillary with 50 cm length (effective length of 10 cm). The separation was achieved by using a background electrolyte composed by 30 mmol/L of 2-(morpholin-4-yl)ethane-1-sulfonic acid and 30 mmol/L of histidine, pH 6.1. The detection limits were 10 and 20 µmol/L and recovery values for spiked samples were 101 and 100% for zinc and ascorbic acid, respectively. The results obtained with the developed procedure were compared to those obtained by titration (ascorbic acid) and flame atomic absorption spectroscopy (zinc), and no statistically significant differences were observed (95% confidence level) [105].

## 5. Conclusion

This literature review represents an up to date survey about all reported methods that have been developed for determination of caffeine, phenylephrine, paracetamol, and ascorbic acid in their pure form, combined form with other drugs, combined form with degradation products, and in biological samples such as liquid chromatography, spectrophotometry, spectroflourimetry, electrophoresis, etc...

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