REVIEW ARTICLE

A REVIEW ON THE ANALYTICAL TECHNIQUES USED FOR THE DETERMINATION OF PARACETAMOL IN PHARMACEUTICAL FORMULATIONS AND BIOLOGICAL FLUIDS

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ABSTRACT
Paracetamol (PM) is an active metabolite of phenacetin and belongs to *p*-aminophenol derivatives. It is also termed as acetaminophen. PM is one of the non-steroidal anti-inflammatory drugs (NSAIDs) which is used as an analgesic and antipyretic agent. It is an OTC drug and is used as a common household analgesic. It is not used as a substitute of aspirin or any other NSAIDs. It is a weak cyclooxygenase (COX) 1 and 2 inhibitor but may inhibit COX-3 in the central nervous system. Many analytical techniques have been used for the assay of PM in pharmaceutical preparations and biological fluids. These techniques include spectrometry, chromatography, spectrofluorimetry, chemiluminescence, electrochemical techniques, flow injection spectrometry and chronoamperometry. In many pharmaceutical preparations PM is also used in combination with other drugs and these techniques have also been used for their simultaneous determination. A detailed review of various analytical techniques used for the assay of PM has been carried out which would be of great help to the chemists and pharmacists involved in the analysis and quality control of drugs.

Keywords: Paracetamol, analgesic, analysis, spectrometry, chromatography.

1. INTRODUCTION
Paracetamol (PM), N-(4-hydroxyphenyl) acetamide (Fig. 1), is a white crystalline powder freely soluble in alcohol, slightly soluble in water and practically insoluble in petroleum ether[1]. It is a weak cyclooxygenase (COX) 1 and 2 inhibitor but may inhibit COX-3 isoenzyme which is a brain specific enzyme and is responsible for the prostaglandin (PG) biosynthesis[2]. When administered orally, it reaches to plasma peak level in 30-60 minutes and its absorption depends on the rate of gastric emptying. It has a fast onset of action and plasma half-life is 2 hrs after the therapeutic dose[3]. Binding to plasma protein is variable but when used with other NSAIDs its binding is reduced. The most common single dose is 1000 mg for three to four times a day[4].

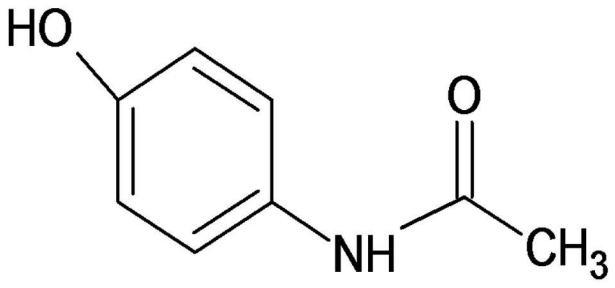

Fig. 1. Chemical structure of paracetamol.

2. ANALYTICAL TECHNIQUES USED FOR THE ASSAY OF PM
Several analytical techniques have been used for the determination of PM and its metabolites in pharmaceutical preparations and biological fluids in order to analyze and to understand the stability of the drug[5]. The details of these techniques are given in the following sections.

2.1. Spectrometric Techniques
2.1.1. Ultraviolet (UV) spectrometry
UV spectrometry has extensively been used for the assay of PM in aqueous solutions and pharmaceutical preparations. A direct spectrometric method has been developed to analyze PM in aqueous medium without using any additive. The absorption of the

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analyte is measured at 243 nm. The study included various other parameters such as the effect of HCl, NaOH, CH_3COOH and NH_3 in absorbance change and spectral shift, time for solution stability, interference of some other analgesics and some polar solvents and temperature. The results showed linearity in the range of 0.3–20 μg/ml with regression of 0.9999 and LOD of 0.1 μg/ml. This method has been used for the determination of PM in pharmaceuticals as well in urine samples[6]. A rapid, accurate and reproducible spectrometric method has been proposed to enhance the aqueous solubility of PM by hydrotropic agents such as sodium salicylate, sodium benzoate, sodium citrate, sodium acetate, urea and nicotinamide. It was found that there was more than 7-folds increase in the aqueous solubility of PM by 10.0 M urea solution as compared to its aqueous solution. This was then analyzed at 245 nm, which showed that the hydrotropic agent and additives did not interfere in the analysis. The method gives good accuracy, precision and reproducibility[7].

A multivariate spectrometric method has been developed for the determination of PM and diphenhydramine or phenylpropanolamine in decongestant tablets. The resolution of these mixtures was obtained by partial least-squares (PLS) regression analysis of the electronic absorption spectral data. In this method the molar ratios of PM with minor compounds were 38:1 and 25:1, respectively, and these have been determined with accuracy and precision. In this method PLS gave some deviation for PM from linearity in the studied concentration range[8]. A new spectrometric method has been developed for the simultaneous determination of salicylamide and PM by H-point standard addition method (HPSAM) and PLS. Both were analyzed in the ratio of 0.2:5–20:1 of salicylamide–PM mixture. The total RSD for the PLS method for 10 synthetic samples in the concentration range of 0–60 μg/ml salicylamide and 0–30 μg/ml PM was 5.1%[9]. The simultaneous determination of ondansetron and PM has been carried out by a simple and reproducible spectrometric method requiring no prior separation. First-order derivative method has been utilized to eliminate spectral interferences. In methanol, ondansetron and PM have λ_{max} at 302 and 246 nm, respectively. The method was found to be linear in the studied concentration range[10].

Simultaneous determination of PM and caffeine has been performed by second derivative-spectrometry. The measurements of PM and caffeine were made at the zero-crossing wavelengths at 260.0 and 288.0 nm, respectively. The calibration graphs were linear for PM and caffeine in the range of 0.1–30.0 mg/l and 0.1–20.0 mg/l, respectively. The LOD for PM was 0.090 mg/l and for that of caffeine was 0.095 mg/l. This method accurately determined both drugs in the ratio of 40:1 to 1:12 w/w[11]. In a spectrometric method aceclofenac (ACE) and PM have quantitatively been determined in bulk and in pharmaceutical preparations. The simultaneous analysis of the two compounds was carried out at 274 and 248 nm. This method shows good accuracy, linearity and precision for the analysis of tablets containing ACE and PM. The result obtained from this method showed good recoveries in the range of 100.49–101.33%[12]. A simple, accurate, precise method for the simultaneous determination of tramadol HCl and PM in two component tablet formulation has been developed by using the concept of internal standard addition. In this method 270.5 and 243.5 nm wavelengths were used for tramadol HCl and PM which showed good linearity in the range of 20–100 μg/ml and 3–15 μg/ml, respectively[13].

The simultaneous determination of meloxicam (MX) and PM by UV spectrometric methods has been performed in combined dosage forms. These methods include simultaneous equation method (Method I), absorbance ratio method (Method II) and correction method (Method III). The wavelengths used for MX and PM in method I were 257.6 and 270.6 nm while in method II, 257.6 nm (λ_{max}) for PM and 297.6 nm (λ_{max}) for MX were used for iso-absorptive point of PM and MX, respectively and for method III 362.0 nm wavelength was used. These methods showed good linearity in the concentration range of 1–5 μg/ml and 7–35 μg/ml for MX and PM, respectively. The percent recoveries of these drugs were near to

100% which indicated that these methods have good accuracy[14]. Two UV spectrometric methods have been developed for the simultaneous estimation of PM and caffeine in pharmaceutical dosage forms. In method A, simultaneous equation method was used using the wavelengths of 243 and 273 nm. Method B involved the formation of Q-absorbance equation at isobestic point (259.5 nm). Both the methods showed good linearity in the concentration range of 2–16 µg/ml for PM and 2–32 µg/ml for caffeine. The methods were validated as per ICH guidelines[15].

A new spectrometric method was introduced in order to determine ibuprofen and PM simultaneously in soft gelatin capsules. In this method simultaneous equations were solved by using two wavelengths at 224.0 and 248.0 nm. This method was found to obey Beer's law and showed good linearity in the concentration ranges of 4–14 µg /ml and 2–12 µg/ml for ibuprofen and PM, respectively. In another spectrometric methods PM and MX were simultaneously determined in pure form and tablet formulations. Method I was based on solving simultaneous equations by absorbance measurement at 256 and 268.8 nm in order to estimate PM and MX, whereas in method II Q-value was determined by absorbance measurements at 308 nm (isobestic point) and at 256 nm (λ_{max} of PM). The results gave good linearity in the concentration range of 5–30 µg /ml[17]. Aspirin and PM have simultaneously been determined in solutions and tablets by a UV spectrometric method. In this method simultaneous equations were solved by the measurement of absorbance at 265 and 257 nm, the ε_{max} for aspirin and PM, respectively. The calibration curve showed good linearity in the concentration range of 2 to 64 µg/ml for both the drugs[18].

A first-order derivative spectroscopy method has been used to analyze MX and PM simultaneously in combined dosage forms. Both drugs were determined quantitatively at 277.47 and 269.44 nm over the concentration range of 10–40 µg/ml and 3–8µg/ml for MX and PM, respectively. The recovery ranges were 99.13–99.42% and 99.45–100.68% with the regression values of 0.9981 and 0.9992 for MX and PM, respectively. The RSD was less than 2.0%[19]. Two methods of simultaneous determination of PM and tolperisone HCl in combined tablet formulations have been developed. In the first method the simultaneous equation method was used by absorbance measurement at 242.5 and 260 nm and in the second method Q-analysis (absorption ratio) was used in which the absorbance equation was used at 254 nm (isoabsorptive point) and 260 nm, the λ_{max} of tolperisone HCl. The linearity was good in the concentration ranges of 4–12 µg/ml and 2–18 µg/ml for PM and tolperisone HCl, respectively. The recoveries were 102.03±3.79 and 98.93±0.90 for method I and 100.4±1.80 and 99.40±1.25 for method II, for PM and tolperisone HCl, respectively[20]. In another method tolperisone HCl and PM were simultaneously determined by first derivative spectrometric zero crossing method in combined dosage forms. This method was used to determine unknown concentrations of the components of interest in a mixture in the presence of interfering substances. The 217.60 nm was the zero cross point for PM which was used for tolperisone and 223.60 nm was the zero cross point for tolperisone that was used for PM. The calibration curve showed linearity in the concentration range of 2–20 µg/ml and 2–12 µg/ml for tolperisone and PM, respectively[21]. By using the same method drotaverine HCl and PM were simultaneously estimated in combined tablet formulations. The wavelengths used were 303.5 nm and 243.5 nm, respectively. The linearity was observed in the concentration range of 550 and 560 µg/ml for drotaverine and PM, respectively. For Q analysis method the two wavelengths were selected at isobestic point (277 nm) and at λ_{max} of PM (243.5 nm) and in first-order derivative method, zero crossing points for drotaverine HCl and PM were selected at 303.5 and 243.5 nm, respectively. Two economical and simple spectrometric methods have been developed for the simultaneous determination of PM and metoclopramide HCl in tablet dosage forms. The first method was based on developing and solving simultaneous equations by using 248.6 and 275.6 nm wavelengths. The second method was absorbance ratio method in which two

wavelengths were selected as isoabsorptive point (265.6 nm) and as λ_{max} of PM (275.6 nm). At selected wavelengths both drugs and their mixtures obey Beer-Lambert law in a given concentration range in both methods[23]. An area under the curve (AUC) method has been employed for the determination of PM and nabumetone. The wavelengths used were 248.8±10 nm (PM) and 269.2±10 nm (nabumetone). Satisfactory linearity relation was found in the concentration range of 5–25 µg/ml for both PM and nabumetone. In the AUC method, the correlation coefficient was 0.9983 for PM and 0.9993 for nabumetone[24].

2.1.2. Visible spectrometry

A rapid determination of PM has been carried out by sequential injection analysis (SIA) in the presence of ammonia on the oxidation of PM by potassium hexacyanoferrate (III) and subsequent reaction with phenol. As a result a blue complex is formed which is measured at 630 nm. The linearity was found to be up to 60 mg/l, RSD was 1.2% where as LOD was 0.2 mg/l[25]. Another SIA kinetic method has been used to determine PM. The oxidation reaction of PM with potassium permanganate in sulfuric acid media has kinetically been investigated. The decrease in absorbance of permanganate is measured at 526 nm. The correlation coefficient of the curve was found to be 0.9931 which is linear at the fixed time of 70 sec at room temperature. The calibration equation "A = 0.0038C + 0.1209" was obtained by adjusting the flow rate at 25 µl/s[26].

For the determination of PM two highly sensitive spectrometric methods have been developed. These methods include the oxidation of PM by iron (III) (method I) and oxidation of *p*-aminophenol after the hydrolysis of PM (method II). Iron (II) then reacts with potassium ferricyanide to form Prussian blue color with a λ_{max} at 700 nm. Linear responses were obtained in the ranges of 1.010 and 0.22.0 µg/ml for method I and method II, respectively. A high sensitivity was recorded for the methods I and II with values of 0.05 and 0.022 µg/ml, respectively. The LOQ of PM by method II and atomic absorption spectrometric method were 0.20 and 0.10 µg/ml, respectively. Intra and inter-day precision was not more than 6.9%. These methods were applied to pharmacokinetic studies by means of salivary samples in normal volunteers who received 1.0 g PM[27].

Spectrometric methods in the visible region have been introduced by Shrestha and Pradhananga[28] in which PM was coupled with 1-naphthol and resorcinol forming azo dyes. These azo dyes follow Beer's law in the range of 0–10 µg/ml of PM at 505 nm. The molar absorptivity and Sandell's sensitivity for the azo dyes coupled with 1-naphthol were found to be 1.68×10^4 l mol^{-1} cm^{-1} and 9.0 ng /ml cm^{-2}, respectively, and with resorcinol were 2.86×10^4 l $mol^{-1}cm^{-1}$ and 5.3 ng /ml cm^{-2}, respectively. The RSD for all samples ranged from 2.2–6.4% at 95% confidence level. The percent recoveries ranged from 97.8–103.4. Another spectrometric method used to analyze PM was by using chromogenic agent (sodium nitroprusside) in a basic solution forming colored *o*-nitrosamines. The λ_{max} and the apparent molar absorption coefficient of the product were 700 nm and 3.4×10^3 L/mol cm, respectively. The linearity range of PM was 0.19–96 µg/ml (r^2=0.9993). The LOD and RSD were 0.10 µg/ml and 0.90%, respectively. The method has been successfully applied to analyze PM in pharmaceuticals as well as in biological samples[29]. A simple spectrometric method has been developed in which PM reacts with iron (III) followed by ferricyanide in an HCl medium to yield a Prussian bluish green colored product. This is measured with maximum absorption at 715 nm. There were no interferences from the common excipients present in the formulations. The results have been statistically compared with the official method. The method has been successfully employed for the determination of PM in various pharmaceutical preparations[30]. A simple and sensitive method for the assay of PM was introduced by Al-Shwaiyat[31]. In this method 18-molybdo-2-phosphate was reduced to form a heteropoly complex. Reaction of 18-molybdo-2-phosphate with PM was found to be very fast at room temperature. The molar absorptivity calculated for PM was equal to that for heteropoly blue formed in the reaction and was

1.15×10^4 l mol^{-1} cm^{-1} at 820 nm measured in the concentration range of 0.3 to 7.5 µg/ml. The LOD was 0.03 µg/ml (l = 5 cm), and the RSD was not more than 2.5%[31].

A method has been developed for the simultaneous determination of PM and *p*-aminophenol in pharmaceutical formulations. This method is based on the introduction of 2,2'-(1,4-phenylenedivinylene) bis-8-hydroxyquinoline (PBHQ) as a novel coupling agent. In this method the microwave assisted alkaline hydrolysis of PM to *p*-aminophenol is carried out, which reacts with the coupling reagent. The λ_{max} of the formed product was found at 650 nm with a molar absorptivity of 3.4×10^4 l mol^{-1} cm^{-1}. The method was found linear in the concentration range of 0.44–5.5 mg/l and the LOD was found to be 0.09 µg/ml.[32]

A new spectrometric method has been developed for the simultaneous kinetic determination of PM and caffeine using H-point standard addition method (HPSAM). The method is based on difference in the oxidation rate of these compounds with Cu (II)-neocuproine system which then formed a Cu(I)-neocuproine complex. This complex was monitored in the presence of sodium dodecyl sulfate (surfactant) at 453 nm (pH 5.0).The linearity was found to be in the concentration range of 1.5–7.0 and 0.1–3.0 µg/ml for PM and caffeine, respectively[33].

2.1.3. Fourier transform infrared (FTIR) spectrometry

A FTIR method has been developed for the in situ control of the crystal size distribution and was then applied to the aqueous crystallization of PM. The solution concentration and the solubility curve of PM in water were determined by using attenuated total reflection (ATR-FTIR) spectroscopy coupled with chemometrics. The metastable zone width of PM was determined using laser back scattering and ATR-FTIR spectroscopy. The in situ chord length distributions of crystals obtained from laser back scattering are related to characteristics of the crystal size distribution. Product crystals where the super saturation temporarily exceeded the metastable limit during operation contained agglomerates and exhibited large size variation. Larger product crystals of more uniform size and shape were obtained from operation where super saturation was successfully controlled to stay within the metastable limit[34]. FTIR and Raman spectroscopic methods were introduced for the identification of orthorhombic (form II) and monoclinic (form I) of PM and for their quantitative determination in mixtures. The intensity ratio (836 cm^{-1} FTIR band to the 806 cm^{-1} monoclinic band) plotted against the inverse monoclinic molar fraction (X) yielded a straight line: $I_{836}/I_{806}=0.515/X+0.700$ and regression was 0.9965. Similarly, the area under the 454 cm^{-1} band in FT-Raman spectra over the area under the 465 cm^{-1} band of monoclinic form is inversely associated to its molar fraction (X): $A_{454}/A_{465}=0.482/X–0.324$ and regression was 0.9954. RSD was <5% for both methods[35].

2.1.4. Chemometric technique

For the chemometric analysis of PM, phenylephrine and chlorpheniramine principal components regression (PCR) and partial least squares regressions (PLS1 and PLS2) were used. At several concentrations the data obtained were found within their linear ranges. The wavelengths used were between 200 to 400 nm. These methods were applied to a tablet formulation and hence found that there was no interference from excipients. Due to the flexibility and mathematical principals PLS1 method showed better results[36].

2.1.5. Flow injection (FI) spectrometry

A FI spectrometric method has been used to determine PM in various pharmaceutical preparations. In this method the oxidation of the analyte in an anionic exchange column with potassium hexacyanoferrate (III) was carried out and then the reaction of the N-(hydroxyphenyl)-p-benzoquinonimine so produced with phenol at room temperature in aqueous ammoniacal solution was monitored. PM concentrations were determined in the range of 0.20–20 ppm with RSD of 0.6% at an injection rate of 42 samples per hour[37]. Another FI method has been proposed for the determination of PM in pharmaceutical formulations. PM is

reacted with sodium nitrite in alkaline medium and the absorbance of the reaction product is measured at 430 nm. The results were compared with those obtained by the official HPLC (United State Pharmacopeia, 2011) method and the relative differences were found from 0.4 to 2.3%, with RSD below 1%[38]. A flow based spectrometry was used to determine PM in pharmaceuticals formulations to prove the usefulness of flow system as an analytical procedure. In this method PM was reacted with sodium hypochlorite forming *N*-acetyl-*p*-benzoquinoneimine which then reacts with sodium salicylate in alkaline medium producing a blue indophenol dye that was measured at 640 nm. The samples were also analyzed by using the AOAC method as a reference method. By applying the paired *t*-test between results no significant difference at the 95% confidence level was observed. Linearity ranged from 5.0–125.0 mg/l (r^2= 0.9992) with a sampling rate of 60 determinations per hr. The LOD and RSD were found to be 0.4 mg/l and 1.5%, respectively[39]. Another spectrometric FI method has been used to determine PM in pharmaceutical formulations. In this method the oxidation of PM by HCl was carried out and the excess of this oxidant was determined by using *o*-tolidine dichloride as chromogenic reagent at 430 nm. The calibration curve was found linear in the concentration range of 8.50×10^{-6}–2.51×10^{-4} mol/l with a LOD of 5.0×10^{-6} mol/l. The RSD was <1.2% for 1.20×10^{-4} mol/l PM solution. The results compared with those obtained by using USP Pharmacopoeia method showed satisfaction at the 95% confidence level[40].

For the simultaneous determination of PM and caffeine a simple UV-photometric flow through biparameter-sensing device has been developed using a wavelength of 275 nm. In this method the C_{18} bonded phase beads were placed into a mini-column just before the flow cell for separation. PM was determined first on sample injected into the carrier solution (deionized water) which easily passed through the mini-column where as caffeine was retained on it. Then, caffeine was easily separated from the precolumn. The analytical signal showed a very good linearity in the range of 10–160 µg/ml and 3.5–50 µg/ml, and LOD was 0.75 and 0.56 µg/ml for PM and caffeine, respectively. If deionized water (pH 12) was used as a carrier solution the ranges were then 25–400 and 4–55 µg/ml with LOD of 2.0 and 0.50 µg/ml, respectively[41].

2.1.6. Spectroflourimetry

PM has directly been analyzed by spectroflourimety in pharmaceutical preparations. The fluorescence measurements (λ_{ex}=333 nm; λ_{em}=382 nm) are performed directly on the powdered sample. The active substance was then diluted in lactose, maize starch, poly (vinylpyrrolidone), talc and stearic acid. Fluorescence intensity was found to be linear dependent on PM concentration within the range of 100–400 mg/g. LOD and LOQ were within the range of 13.0–16.7 and 43.1–55.7 mg/g for samples with different ingredient proportions. RSD for all tested ingredients proportions was <2.7%. The proposed method was compared with the British Pharmacopoeia (2016) method and results showed no statistical difference at the 95% confidence level[42]. A selective spectrofluorimetric method has been designed for the determination of PM in tablets. This important technique can be characterized by its sensitivity, simplicity, clarity and cheaper cost than the current official methods. The employed methodology involves coumarinic compound formation obtained by reaction between paracetamol and ethylacetoacetate (EAA) in the presence of sulphuric acid as a catalyst. The reaction product is highly fluorescent at 478 nm, being excited at 446 nm. The linear concentration range of the application was 0.1–0.4 µg/ml of paracetamol and the detection limit was 57 ng/ml. The influence of different variables was studied and optimized through chemometric techniques[43]. A new spectro-fluorometric method has been developed for the determination of phenylephrine (PHE) and PM (large amount) in tablets. In this method standard addition of phenylephrine to a slightly acid aqueous solution (HCl) of the tablet components was used. The LOD and LOQ were found to be 0.08 and 0.27 mg/l, respectively[44].

2.2. Chromatographic Techniques

2.2.1. Thin layer chromatography (TLC)

PM has quantitatively been determined by TLC in bulk powder and pharmaceutical dosage forms in the presence of its degradation product. In this method PM was dissolved in methanol and then the solution spotted on a thin layer plate of silica gel G_{254}. The mobile phase, ethyl acetate: benzene: acetic acid in a ratio (1:1:0.05, v/v/v) was used. Absorbance was measured at 250 nm. Calibration curves showed the concentration in the range of 5–20 µg/spot. Spectrodensitometry was used to scan TLC plates for quantitation by comparing the area under the peaks obtained. The results were statistically compared with those obtained by applying a reference method[45]. For the identification and quantification of PM in different pharmaceutical commercial brands adsorption thin layer chromatography with densitometry has been introduced. This separated PM from 4-aminophenol and 4'-chloroacetanilide. The UV densitometry was conducted at 218 nm. The proposed method was validated and compared with a pharmacopoeial UV-spectrometric method and the results showed statistically that this method could be used as a substitute method[46]. The simultaneous determination of PM, chlorzoxazone (CZ) and their toxic impurities, 4-amino phenol (4AP) and 2-amino-4-chlorophenol (2ACP) has been carried out by a highly sensitive, selective and accurate validated TLC–densitometric method. Chloroform-methanol-glacial acetic acid (9.5:0.5:0.25, v/v/v) was used as the developing system. The TLC plate used was pre-activated silica gel 60 F_{254} and 225 nm was the detection wavelength. Calibration curves were constructed by using polynomial equation in the ranges of 0.3–3, 1–10, 0.06–3 and 0.04–3 mg/band for PM, CZ, 4AP and 2ACP, respectively. The results were statistically compared with the reported RP-HPLC method using F-test and Student's *t*-test[47].

2.2.2. High performance thin layer chromatography (HPTLC)

A HPTLC method has been developed for the simultaneous determination of PM and piroxicam (PX) in tablet dosage forms. The spots of separated drugs were determined by densitometry (288 nm). The solvent system was n-dichloroethane: methanol:triethylamine (10:2.5:1, v/v) and TLC sheets used were of silica gel 60 F_{254}. The calibration curves were linear in the range of 1.625–14.625 µg/spot and 0.1–0.9 µg/spot for PM and PX, respectively[48].

Baheti al[49]. developed a sensitive, selective, precise HPTLC method for the simultaneously analysis of PM and etoricoxib in bulk and tablets. The separated spots were measured at 258 nm. The calibration curves were found to be linear in the concentration range of 100–600 ng/spot and 200–1200 ng/spot with regression coefficients of 0.998 and 0.995 for PM and etoricoxib, respectively. The results of recovery were in the range of 98–102%.

2.2.3. Micellar electrokinetic chromatography (MEKC)

A new MEKC method has been developed for the simultaneous determination of PM and chlorpheniramine maleate (CPM). In this method separation was achieved in 25.5 min by using sodium dihydrogenphosphate-sodium tetraborate buffer (10 mM, pH 9.0) having sodium dodecyl sulfate (SDS) (50 mM) and acetonitrile (26% v/v) in it. The detection was carried out by a diode array detector at 214 nm. The calibration curve was found to be linear in the range of 10–250 µg/ml (r^2= 0.999). Recoveries were 99% with LOD of 0.4 and 0.5 µg/ml and LOQ of 2 and 4 µg/ml and RSD of 3.1 and 2.4%, for PM and CPM, respectively[50].

2.2.4. High performance liquid chromatographic (HPLC)

The separation of PM and its four major metabolites (glucuronide, sulfate, cysteine and mercapturate conjugates) in mouse plasma samples has been achieved by a reversed phase ion pair HPLC method. The mobile phase consisted of an aqueous solution of 0.01 M tetrabutylammonium chloride and tris buffer (pH 5.0) with phosphoric acid, and methanol as the organic solvent. In the ODS column initially 30% methanol was used for gradient elution. After 0.5 min the methanol concentration was increased

to 75% over 7.5 min. A methanol solution of theophylline, an internal standard, was added to the mouse plasma sample, centrifuged and immediately injected into the chromatographic system. The advantages of this method include good and rapid separation (last metabolite detected at 6.86 min); well resolved peaks[51]. A RP-HPLC method has been developed for the determination of PM in pharmaceutical formulation at 193 nm. A C_{18} stationary phase is used at the flow rate of 1.78 ml/min with a methanol-water (1/2, v/v) mixture as mobile phase where sulfamethoxazole is used as an IS. The validation of the method showed good linearity, precision and reproducibility[52]. The qualitative and quantitative determination of PM in different body fluids, e.g. blood, urine, cerebrospinal fluid, synovial fluid, vitreous humor, and in tissue samples is carried out by a solid phase extraction method. In this HPLC method body fluids were mixed with phenacetine (IS) and phosphate buffer (pH 6.8) then protein was precipitated using acetonitrile. Pre-conditioned bakerbond C_{18} SPE column was used for the supernatant which was obtained after strong centrifugation. Good recovery rates were showed by elution with methanol. The extracts were then analyzed with ultraviolet detection with good results[53].

A stability-indicating RP-HPLC method has been developed by Bhimavarapu et al.[54] to determine PM in pharmaceutical formulations. For the investigation of the stability of the drug the samples were forced degraded under different conditions as given by International Council on Harmonization (ICH). The sample obtained was used to develop a stability-indicating HPLC method for PM. A C_{18} column was used for the chromatographic separation of PM and its degradation products. The mobile phase containing a mixture of acetonitrile and methanol in ratio of 60:40 (v/v) with the flow rate of 1 ml/min was used and detection was carried out at 230 nm. A new HPLC method has been developed for the simultaneous determination of PM and caffeine in a tablet dosage form. The column used in this method was C_{18} at a flow rate of 1.5 ml/min with detection at 220 nm. The solvent system consisted of 1 mM phosphate buffer (pH 3.0) and acetonitrile (85:15 v/v) with 0.2% triethylamine (v/v). The method was linear in the concentration range of 31.25–250 µg/ml (r = 0.9999) and 4.06–32.50 µg/ml (r^2 = 0.9986) for PM and caffeine, respectively. Mean recoveries for PM and caffeine were 99.37 and 99.12%, respectively[55]. A simple RP-HPLC method has been developed for the simultaneous determination of PM and ascorbic acid in tablet formulations. A C_{18}, 5 µm column was used for the separation at a flow rate of 1.0 ml/min with detection at 254 nm. The mobile phase used was 1 mM sodium pentane sulfonate in a mixture of formic acid (0.4 ml), methanol (25 ml) and water (75 ml). The retention times for ascorbic acid and PM were 3.53 and 6.09 min, respectively. The mean recoveries were 99.16 and 98.76%, respectively[56]. In another RP-HPLC method, simultaneous determination of PM and ibuprofen in combined pharmaceutical formulations has been achieved. The separation was carried out on a C_{18} column with methanol and 0.05 M sodium dihydrogen phosphate (65:35, v/v) as mobile phase at a flow rate of 1.0 ml/min and detection at 230 nm. The separation was achieved in <8 min. The calibration curves were found to be linear in the concentration range of 50.00–400.0 µg/ml and 20.00–160.0 µg/ml for PM and ibuprofen, respectively. The RSD was not more than 0.62% whereas the recoveries were in the range of 99.53% and 99.83%, respectively[57].

The simultaneous determination of MX and PM has also been carried out by RP-HPLC in bulk and in dosage forms. In this method a Hypersil ODS C_{18} column was used with methanol and water as mobile phase (70:30, v/v) at a flow rate of 0.6 ml/min and detection at 240 nm. Retention times were 3.5 min and 5.0 min for MX and PM, respectively. The method was found to be linear with regression of 0.939 and 0.996 for MX and PM, respectively[58]. A RP-HPLC method for the simultaneous determination of PM and codeine phosphate has been developed and validated. A RP-18 column was used for the separation and the mobile phase was a mixture of acetonitile with buffer solution (pH=2.5) (15:85, v/v) and a flow rate of 1.0 ml/min with detection at

210 nm. The linearity ranges were 100–1000 µg/ml and 6–60 µg/ml, for PM and codeine phosphate, respectively and recoveries were in the range of 99.88–100.2% and 99.33–100.3%, respectively[59].

2.2.5. Gas chromatography–mass spectrometry (GC–MS)

PM and tramadol HCl (TR) in a binary mixture have simultaneously been determined by using HPLC–UV and GC–MS techniques. In HPLC a Hypurity Advance column was used with a mixture of mobile phase consisting of phosphate buffer (pH 6.3) and acetonitrile (90:10, v/v). PM and TR were detected at 220 nm. In GC–MS a 100% dimethylpolysiloxane (Rtx-1) column with temperature programming was used to separate PM and TR. The EI mass spectra of PM and TR were characterized by $[M]^+$ at 151 (base peak at *m/z* 109)and $[M]^+$ at 263 (base peak at *m/z* 58). By using HPLC method the calibration curves were found to be linear in the range of 10–400 µg/ml for both PM and TR while for GC-MS method the ranges were 75–500 and 25–350 µg/ml for PR and TR, respectively. In the presence of common pharmaceutical adjuvant no interference peaks were observed[60].

2.3. Chemiluminescence (CL) Technique

This technique has been used for the analysis of PM in pharmaceutical and biological samples. PM was indirectly detected by using a capillary electrophoresis chemiluminescence (CE–CL) detection system which involved an inhibitory effect on a luminol-potassium hexacyanoferrate(III) ($K_3[Fe(CN)_6]$) CL reaction. PM is passed through the separation capillary where it is mixed with luminol incorporated with the running buffer. After the whole process PM inhibits the CL reaction and an inverted PM peak can be detected, which shows that the degree of CL suppression is proportional to PM concentration. Maximum CL signal was observed within an electrophoretic buffer of 30 mM sodium borate (pH 9.4) containing 0.5 mM luminol and an oxidizer solution of 0.8 mM $K_3[Fe(CN)_6]$ in 100 mM NaOH solution. Results showed a linear relationship ranging from 6.6×10^{-10}–6.6×10^{-8} M with the regression of 0.9999, and LOD of 5.6×10^{-10} M for PM. The RSD of the peak area for 5.0×10^{-9} M of PM was 2.9%[61].

2.4. Electrochemical Techniques

A new electrochemical method has been applied for the detection of PM and 4-aminophenol. This method involved the application of a sensitive micro-fluidic paper-based device with electrochemical detection. The separation channels (2.0 mm) were created on paper using a wax printing process to define the regions of the device. The base line was achieved in 0.1 mol/l acetate buffer solution (pH 4.5) at 12 mm from the working electrode and by applying a potential of 400 mV versus pseudo Au on the working electrode. The separated analytes were detected at the end of the hydrophilic separation channel. The LOD was found to be 25.0 and 10.0 µmol/l for PM and 4-aminophenol, respectively[62].

2.4.1. Voltammetry

A low-cost electrochemical flow-through cell was designed to use in combination with FI system. This detector cell used acentrosymmetric radial flow thin-layer geometry with a stainless steel auxiliary electrode and a reference electrode (Ag/AgCl) without a salt bridge. The 5H pencil lead electrode used as a working electrode instead of glassy carbon electrode. Appropriate volume of sample and standard solution of PM were injected into the FI system with an optimum flow rate of 1 ml/min. The cyclic voltammograms were recorded in the range of –0.5 to +2.0 V with a rate of 40 mV s^{-1}. Linear calibration curve in the range of 0.1–5 mM PM was obtained with a correlation coefficient of 0.9964. This method has been applied to eight commercial pharmaceutical preparations in order to determine PM in which the recoveries of four tablet samples ranged from 103–112% with RSD of 0.1–1.3%[63]. Goyal and Singh[64] carried out voltammetric determination of PM at C60-modified glassy carbon electrode in a wide concentration range. The common biological and chemical additives did not show any interference. A linear calibration curve was obtained in the range of 0.05–1.5 mM (r^2=0.985) and the sensitivity of the method was 13.04 µA mM^{-1}. Sweep rate studies indicated that the electrode reaction is

followed by follow-up chemical reactions. The proposed method was rapid and has been applied to the determination of PM in different tablets and urine samples with several advantages over other analytical methods. The SD was 5.53% for eight determinations. The electrochemical behavior of PM on grapheme-modified glassy carbon electrodes (GCEs) has been investigated by cyclic and square-wave voltammetry. This method was used for the quantitative determination and screening of PM. The results showed that the graphene-modified electrode exhibited excellent electrocatalytic activity to PM. At the modified electrode a quasi-reversible redox process of PM was observed and as a result the over-potential of PM decreased significantly which was then compared with that at the bare GCE. The results showed LOD of 3.2×10^{-8} M, recovery ranges from 96.4–103.3% with RSD of 5.2%[65]. Another cyclic voltammetry method has been introduced in which an electrochemical sensor was developed to detect PM by electrochemically co-depositing glutamic acid and gold nanoparticles on a single-walled carbon nanotube film (AuNP-PGA/SWCNT). This indicated that the electrochemical oxidation of PM at the AuNP-PGA/SWCNT film electrode involved a two-electron, one-proton process and was pH dependent. Different pulse voltammograms gave a well defined oxidation peak at 360 mV in 0.1 M phosphate saline buffer (pH 7.2). A linear calibration curve was obtained from 8.3–145.6 µM with correlation coefficient of 0.997. The LOD was found to be 1.18 µM. The reproducibility ranged from 1.15–5.21% with RSD of 3.56%. The AuNP-PGA/SWCNT film electrode was able to detect PM in the presence of ascorbic acid, two well-defined oxidation peaks were detected one for ascorbic acid at 0.15 V and the other for PM at 0.39 V. The results indicate that the film is useful for accurate detection of PM[66].

Cyclic voltammery has been used to investigate the electrochemical behavior of PM at a graphene-modified carbon paste electrode in an ammonium buffer solution (pH 8.5). As compared to the bared electrode this electrode showed excellent electrocatalytic activity towards the oxidation and reduction of PM as the peak potential lowers and peak current was improved remarkably. Also a quasi-reversible redox process at the electrode was observed for PM with a peak separation of 66 mV at a scan rate of 50 mV^{-1}. By using the graphene modified carbon paste electrode, square wave voltammetry was applied to the quantitative determination of PM after an accumulation time of 2 min. The results showed that the calibration graph was linear in the concentration range of PM of 2.5–143 µM with a sensitivity of 0.282 µA/µM and LOD of 0.6 µM[67].

A new selective square-wave voltammetry method has been developed for the simultaneous determination of PM and penicillin V on a bare (unmodified) boron-doped diamond electrode. The method has good potential separation between the oxidation peak potentials of both drugs (0.35 V) in a mixture. The cyclic voltammetry showed that PM gave a quasi reversible wave and penicillin V provided an irreversible oxidation peak. The oxidation peak of PM and penicillin V shows a good linearity within the concentration range of 0.4 to 100 µM. The LOD was found as 0.21 and 0.32 µM and the RSD was 1.5 and 2.1% for PM and penicillin V, respectively[68].

2.5. Chronoamperometry

For the chronoamperometric determination of PM a biosensor based on vaseline/graphite modified with avocado tissue (Persea americana) as the source of polyphenoloxidase has been developed and used in pharmaceutical formulations. PM was oxidized to N-acetyl-p-benzoquinoneimine by these enzymes whose electrochemical reduction back to PM was obtained at a potential of –0.12 V. For the accumulation of N-acetyl-pbenzoquinoneimine at the electrode surface PM reference solutions were added in glass cell and then the current response was monitored by 120 s. The currents obtained at 70 s were proportional to the PM concentration from 1.2×10^{-4} to 5.8×10^{-3} mol/l with a LOD of 8.8×10^{-5} mol/l (r^2=0.9927). The recovery of two samples ranged from 97.9–100.7% and a RSD lower than 0.5% for a solution containing 5.0×10^{-3} mol/l PM in 0.10 mol/l phosphate buffer solution (pH 7.0)[69].

3. CONCLUSION

The application of different analytical techniques (spectrometric, chromatographic, electrochemical) in the analysis of PM in pure solutions, pharmaceutical formulations and biological fluids, alone or in combination with other drugs and degradation products/metabolites have been presented. Each technique has its own importance and advantage depending in the nature of the sample and quantity of the drug. Spectrophotometric and chromatographic methods are most sensitive and can determine the drug in the ng range. However, HPTLC and HPLC have the advantage of determining the drug in the presence of its degradation products and metabolites with good accuracy and precision.

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