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# Recent Applications of Analytical techniques for counterfeit drug analysis: A Review

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**Abstract:** Drug counterfeiting has exaggerated dramatically over the past decade causing a significant threat to human health. It must be processed in regard to medicines and the matter isn't restricted to a violation of belongings rights, however clearly involves every kind of poor quality pharmaceutical merchandise, including falsified and substandard medicine. The present paper discuss the review of the current quantitative analytical procedures which are used for counterfeit drug detection. The analytical techniques employed like ultraviolet/ visible spectrophotometer, fluorimetry, titrimetry, electro analytical techniques, chromatographic methods (thin-layer chromatography, gas chromatography and high-performance liquid chromatography, Ultra performance high pressure liquid chromatography), capillary electrophoresis and vibrational spectroscopies are discussed for the quantitative analysis of pharmaceutical counterfeit.
**Keywords:** Pharmaceutical merchandise; Substandard medicine; chromatography.

## 1.INTRODUCTION:

A counterfeit medication or a counterfeit drug could be a medication or pharmaceutical product that is produced and sold-out with the intent to deceivingly represent its origin, legitimacy or effectiveness. A counterfeit drug could contain inappropriate quantities of active ingredients, or none, is also improperly processed in the body (e.g., absorption by the body), could contain ingredients that aren't on the label (which could or might not be harmful), or is also provided with inaccurate or faux packaging and labeling. It is believed that approximately 20% of all pharmaceutical products sold worldwide are counterfeits, representing a $50 billion/year industry. For this reason, appropriate methods of drug detection (qualitative and quantitative analyses, purity testing, chiral separation, related substance and stoichiometric determination) are of great importance to the analyst[1]. In the present work we reviewed some of the recent quantitative published analytical methods and their applications in pharmaceutical analysis, mainly from 2000 to 2013.The chemical analysis of counterfeit drugs poses a challenging task in terms of sample throughput, dynamic range and identification of unknowns. As per the review, The selected papers were organized according to the analytical technique employed. Several techniques like ultraviolet/visible (UV/VIS) spectrophotometry, fluorimetry, titrimetry, electro analytical techniques (mainly voltammetry), chromatographic methods (thin-layer chromatography (TLC), gas Chromatography (CG) and mainly high performance liquid chromatography (HPLC)), capillary electrophoresis (CE), vibrational spectroscopies, raman spectroscopy and hyper spectral imaging are the main techniques that have been used for the quantitative analysis of pharmaceutical spurious compounds[2]. While infrared (IR) spectroscopy can primarily detect non-genuine features on the packaging and identify some of the compounds present in the samples, only by spectra inspection, mass spectrometry allows the study of impurity profiles related to the API and often the detection of known substitute API's.

On the other hand, imaging is very helpful for scrutiny a collection of tablets and notice chemical and physical patterns within the tablets which distinguish between real and counterfeit, in addition as inside counterfeit (potentially allocating similar tablets to an equivalent source), because it provides quick abstraction and spectral informations and needs no sample preparation ,are the most useful techniques that are used for the pharmaceutical counterfeit detection. Counterfeit detection is thus the primary crucial step aiming at the removal of counterfeit medication from the market. However, very little effort has been placed on the chemical identification and quantification of the elements in these medication, rather than only detecting their non-authenticity. Identifying and quantifying the composition of a counterfeit tablet falls into the ultimate purpose of detecting the source and cracking down the illegal operation. The identification of chemical compounds showing a restricted geographical distribution.


**Figure 1** Photographs of a 1) fake and 2) original Avastin packaging.

## 2 .ANALYTICAL METHODS FOR PHARMACEUTICAL COUNTERFEIT ANALYSIS

### 2.1 UV/VIS Spectrophotometric methods

Several UV/VIS spectrophotometric tests are widely developed for analyse counterfeit medication. As most prescription drugs possess chromophores groups, they will be determined directly within the ultraviolet region while not the necessity for a derivatization reaction. However, direct UV/VIS is not suitable for simultaneous determination of drugs with spectral overlapping[3]. For this reason, derivative spectrophotometry offers an alternative approach to the enhancement of sensitivity and specificity in mixture analysis. This technique has been frequently used to extract information from overlapping bands of the analyte sand interferences. It consists of calculating and plotting one of the mathematical derivatives of a spectral curve[4]. Raman spectroscopy has been used to detect counterfeit Viagra by de Veij et al.[5], counterfeit Cialis by Trefi et al.[6].

Jacques O.De Beer et al introduced a comparision and combination spectroscopic method for detection of erectile dysfunction class of counterfeit drugs. They used Raman, NIR and FT-IR spectroscopy for this purpose. In this study , 55 counterfeit and imitations of Viagra, 9 genuine Viagra, 39 counterfeit and imitations of Cialis and 4 genuine Cialis were analysed by Raman, NIR and FT-IR [7].

P.de Peinder et al published a method based on comparision of NIR and RAMAN spectroscopy for detection of Lipitor (Atorvastatine) in combination with chemomerics.Due to fast and non-destructive capability ,NIR is very economical and widely used. NIR is not always highly substance specific. Raman spectroscopy has been applied as an alternative tool. Partial least sqare discriminant analysis (PLS-DA) models have been generated for both NIR and Raman spectra [8].

El-Gindy, Emara, and Shaaban reported a chromatographic and two chemo metric methods for the analysis of two ternary mixtures containing drotaverine hydrochloride (DR) with caffeine and paracetamol (mixture 1) and DR with metronidazole and diloxanide furoate (mixture 2).The chemometric methods applied were PLS and PCR. These approaches were applied using information included in the UV absorption spectra of appropriate solutions in the wavelength range of 210–300 nm with 1 nm intervals. Calibration of PCR and PLS models was evaluated by internal validation, by cross validation and by external validation. The authors showed that the proposed methods were successfully applied for the determination of the two ternary combinations in laboratory-prepared mixtures and commercial tablets. Besides, according to the authors, the results of PLS and PCR methods were compared with the HPLC method, and a good agreement was found. All methods show adequate sensitivity for all analytes (detections limits less than 1.98 x 10-1μg mL-1). This paper also presents a novelty, because no United States, British, or European compendia analytical method has been reported for the simultaneous determination of the five drugs in the two studied ternary mixtures [9].

Storlarczyc and coworkers reported first and second order derivative spectrophotometric methods for individual determination of fluphenazine, pernazine, haloperidol and promazine using methanol as solvent at two wavelengths. The authors concluded that no interference of matrix constituents was observed and that the developed method can be useful for quality control of pharmaceutical formulations and it is an option for commonly used expensive chromatographic methods. This method proved satisfactory, although poor sensitivity for all drugs (detections limits less than 3.15 μg mL-1). The USP[11] and the Britishpharmacopoeia BPi[2] describe methods for analysis of these drugs in tablets as titrimetry, direct and derivative spectro photometry and HPLC. Therefore, this work does not present a novelty from an analytical point of view [10].

## 2.2 Fluorimetric methods

Luminescence spectroscopy is an analytical method extremely sensitive and has been widely applied in solving problems that require low detection limits[13]. This technique is applied for the analysis of concentrations in the ng mL-1 range and, sometimes, in the pg mL-1 range in complex matrices[14].Thus, measurements of luminescence intensity have allowed the selective and sensitive quantitative determination of a variety of active pharmaceutical ingredients. However, despite all the advantages of luminescent methods, only certain classes of compounds exhibit luminescence native, as a consequence of the deactivation processes occurring in a molecule. Moreover, the effects of scattering and absorption limit the use of luminescent methods when compared to other analytical methods such as chromatography and UV/visible spectrophotometry[13]. Luminescence spectroscopy can be applied using three different processes: fluorescence, phosphorescence or chemiluminescence. However, fluorimetry is the most commonly luminescence method applied in pharmaceutical analysis. Therefore, below are described relevant examples of fluorimetric methods applied to analysis of pharmaceuticals.

Abdel and Shaalan published spectrofluorimetric and spectrophotometric technique for the determination of pregabalin (PRG). In the spectrofluorimetric method, PRG was reacted with fluorescamine. The optimum conditions were established and the spectrofluorimetric method was applied to the determination of the drug in capsules. According to the authors, no interference could be observed from the additives listed in capsules. Moreover, the spectrofluorimetric method was extended to the in-vitro determination of pregabalin in spiked urine and interference from endogenous amino acids could be eliminated through selective complexation with copper acetate and co-administered drugs such as chlordiazepoxide, clonazepam, diazepam, nitrazepam and lamotrigine did not interfere with the assay. Also, the concentration range was 20-280 ng mL-1 for spectrofluorimetric method and 1-8 μg mL-1 for spectrophotometry. These results show that the spectro fluorimetric method is more sensitive and reliable than the mentioned spectrophotometric methods for determination of this drug [15].

El-Enany and coworkers developed a second derivative fluorimetric method for combined analysis of binary mixture of chlorzoxazone (CLZ) and ibuprofen (IP). Because CLZ and IP exhibit native fluorescence, the method described by the authors was based on measurement of the synchronous fluorescence intensity of these drugs in methanol. However, both the excitation and emission spectra of CLZ and IP overlapped. Then, a second derivative fluorescence spectrum of CLZ and IP was derived from the normal synchronous spectra. The different experimental parameters affecting the fluorescence of the two drugs (Nλ selection, pH, type of the diluting solvent, stability time and ionic strength) were optimized. The authors proved that the high sensitivity attained by the proposed method allowed the determination of both drugs in their coformulated dosage forms and biological fluids and real human plasma samples. USP [11] recommends spectrophotometric method for determination of CLZ in pure form and HPLC method for its determination in tablets. For IP, BP[12] recommended direct titration for analysis of raw material, while USP[11] recommends an HPLC technique. However, a method for simultaneous analysis of these drugs does not mention in pharmacopoeias, which shows an analytical advantage of the fluorimetric method. Besides, this method showed a high sensitivity with low quantization limits (0.85 and 0.03 μg mL-1 for CLZ and IP, respectively)[16].

Walash and coworkers published two methods for the determination of rosiglitazone maleate (ROZ) in pure form, pharmaceutical preparations, and biological fluids. Method I was a spectrophotometry method. Method II was based on the spectrofluorimetric determination of ROZ through complex formation with Al+3 in acetate buffer of pH 5. The relative fluorescence of the drug was measured at 376 nm after excitation at 318 nm. Both methods were applied for the determination of ROZ in tablets. Furthermore, method II was applied for the determination of ROZ in spiked and real human plasma. The stability of the formed complexes in both methods was studied by the authors, and the proposed methods were found to be stability indicating ones. No United States[11], British[12] or European[18] compendia analytical method has been reported for analysis of ROZ. Moreover, the quantification limit of the spectrofluorimetric method was 0.02 μg ml-1 demonstrating the sensitivity of this technique to human plasma applications, making it attractive to laboratories lacking sophisticated separation techniques[17].

Walash and coworkers published akinetic spectrofluorimetric method for the individual determination of verapamil hydrochloride, diltiazem hydrochloride, nicardipine hydrochloride and flunarizine using water as diluting solvent. The method was based on oxidation of the studied drugs with cerium ammonium sulphate in acidic medium. The fluorescence of the produced Ce was measured at 365 nm after excitation at 255 nm. The different experimental parameters affecting the development and stability of the reaction product (Ce concentration, type of acid and its concentration, heating time, temperature and diluting solvents) were individually optimized. The method was applied to the analysis of commercial tablets and the authors concluded that the proposed method is simple, rapid and inexpensive. Both BP[12] and USP[11] recommend non-aqueous titration for analysis of verapamil hydrochloride raw material, the USP describes a HPLC method for analysis in tablets and the BP recommends spectrophotometric method for its formulations. Regarding diltiazem hydrochloride, the BP recommends HPLC for related substances, non-aqueous titration for assay of raw material and the USP recommends HPLC method for the raw material and for its formulations. Nicardipine and flunarizine are not yet listed in Pharmacopoeias, making the proposed method more attractive for the later two drugs. Also, the method showed good sensitivity for all drugs [19].

Marques, da Cunha and Aucelio developed a fluorimetric method to quantify camptothecin (CPT) in irinotecan (CPT-11) and in topotecan (TPT) based anti-cancer drugs. For samples containing TPT, detection was made at 368 nm; whereas, in samples containing CPT II, the detection was made at 267 nm isodifferential wavelength, using the second derivative of the synchronized spectrum. The authors concluded that in practical terms, determinations using spectrofluorimetry were made in a faster, cheaper, and simpler way when compared to the ones made using HPLC. This work is relevant because no USP [11] , BP[12] , or European[18] Pharmacopoeia (EP) has been reported a method for the determination of these drugs. Besides, the method was sensitive, with limits of detection of about 9 ng mL-1, making this method an interesting alternative to chromatographic techniques[20].

Omar developed spectrophotometric and spectrofluorimetric methods for the determination of hydrochlorothiazide, indapamide and xipamide based on ternary complex formation with eosin and lead in the presence of methylcellulose as surfactant. The fluorescence method was investigated for the purpose of enhancing the sensitivity of the determination. According to the author, both methods have been fully validated and successfully applied for the determination of the studied drugs in their pharmaceutical tablets. Moreover, common excipients used as additives in tablets do not interfere with the proposed methods. The USP [11] describes HPLC method for hydrochlorothiazide raw material and for tablets. BP [12] recommends titration for raw material and spectrophotometry for tablets. Regarding indapamide, both USP and BP recommend a HPLC method for raw material and for tablets. No United States, British or European compendia analytical method has been reported for ximpamide. The positives of these methods are the simplicity (prior extraction is not necessary), good sensitivity (quantitation limits less than 0.15 μg mL-1), and possibility of application to pharmaceutical tablets [21].

Rahman, Siddiqui and Azmi developed a spectrofluorimetric method for the determination of doxepin hydrochloride in commercial dosage forms. The method was based on the fluorescent ion pair complex formation of the drug with eosin in the presence of sodium acetate–acetic acid buffer solution of pH 4.50. The extracted complex showed fluorescence intensity at 567 nm after excitation at 465 nm. According to the authors, the method has been successfully applied to the determination of doxepin hydrochloride in commercial dosage forms. USP [11] recommends HPLC method for determination of doxepin hydrochloride in pure form and capsules. The BP [12] recommends titration for assay of doxepin hydrochloride raw material and there is no monograph for its formulations. This method has the advantage of having simple operation and high sensitivity (limit of quantitation of 8.90 ng mL-1) [22].

The spectrofluorimetric methods cited in this paper have the advantage of being highly sensitive in comparison with spectrophotometric methods and even when compared with chromatographic techniques. Moreover, due the fact that few molecules present luminescence native, these methods are considerably selective, allowing the quantitation of some drugs in biological matrices. However, the limitations of this technique are the possibility of analyzing only luminescent compounds or the need for derivatization reactions, which make the analysis more complex and time consuming. Another interesting factor is that fluorimetric methods generally do not allow the analysis of related substances, limiting this technique in quality control applications.

### 2.3 Titrimetric methods

Although titrimetry has already been extensively used in past years, a literature search reveals that there are few recent methods that employ this technique for analysis of pharmaceuticals. Some of the advantages of titrimetry are short time of analysis and low cost of equipment required. However, titrimetric methods show lack of selectivity when compared to modern separation techniques such as HPLC or capillary electrophoresis, which probably led to the current disuse of titrimetric techniques. Below are listed some recent applications of this technique in pharmaceutical analysis.

Basavaiah and coworkers developed and optimized four methods for the determination of stavudine (STV) in bulk drug and in dosage forms by titrimetry and spectrophotometry. In titrimetry, aqueous solution of STV was treated with a known excess of bromate-bromide in HCl medium followed by estimation of unreacted bromine by iodometric back titration. Calculations in titrimetry were based on a 1:0.666 (STV: KBrO3) stoichiometry. According to the authors, the methods when applied to the determination of STV in tablets and capsules were found to give satisfactory results. USP[11] and BP[12] recommend HPLC method for assay and related substance of STV in pure form. Also, USP recommends HPLC method for its formulations. Although the titrimetric method is simple, rapid and cost-effective, it does not have analytical advantages over HPLC and spectrophotometric methods, when considering the sensitivity and specificity. However, the titrimetric method was able to quantify STV in drug formulations, which may not be possible in other cases by using this simple analytical technique [23].

Mostafa and AlGohani described a spectrophotometric method and two titrimetric methods for the determination of nordiazepam. According to the authors, these methods depend on the reaction of nordiazepam with potassium bismuth iodide which give an orange precipitate. Determination of nordiazepam by titrimety in the precipitated complex is done iodometrically using standard potassium iodate solution or complexometrically using standard EDTA solution and xylenol orange indicator. The proposed methods were applied by the authors for the determination of nordiazepam in tablets and the validity of the proposed methods was assessed by applying the standard addition technique. No USP[11], BP[12], or EP [18] has been reported a method for the determination of nordiazem. Therefore, the presented methods, although simple, can be used in analytical laboratories that have limited resources [24].

Rajendraprasad, Kanakapura and Vinay developed two titrimetric methods for the determination of hydroxyzine dihydrochloride (HDH) in pure form and in tablets. The methods were based on acid–base reactions in which the hydrochloride content of the drug was determined by titrating with an aqueous standardized NaOH solution either visually using phenolphthalein as indicator or potentiometrically using glass-calomel electrode system. The procedures were also applied for the determination of HDH in its dosage forms and, according to the authors, the results were found to be in good agreement with those obtained by the reference method. USP [11] method for assay and related substance for this drug in pure form and its formulations is by HPLC. BP [12] recommends potentiometric titration for assay of raw material. Therefore, this method, although it was applied in tablets, presents no novelty from an analytical point of view[25].

Ramesh and coworkers described one titrimetric and two spectrophotometric methods for the determination of doxycycline hyclate (DCH) in bulk drug and in its formulations. In titrimetry, DCH was treated with a known excess of bromatebromide mixture in acid medium and the residual bromine is back titrated iodometrically after the reaction between DCH and in situ bromine. The authors concluded that the proposed titrimetric procedure is far simpler than the published methods since it is free from critical working conditions and does not use any expensive instrumentation. USP [11] and BP [12] methods for assay and related substance for this drug in pure form and its formulations are by HPLC. The authors compared statistically the obtained results with the official BP method and no significant difference existed between the proposed methods and the reference method. Furthermore, the authors compared the sensitivity of the methods with those reported in the literature and show that the proposed methods are one of the most sensitive ever reported for DCH. So, the proposed methods are interesting alternatives to laboratories that do not have HPLC equipments [26].

De Sousa and Cavalheiro described a titrimetric procedure for the determination of minoxidil using $KMnO_4$ as oxidizing agent. The best conditions were optimized considering the $H_2SO_4$, $KMnO_4$ and minoxidil concentrations, the temperature of the system and the order of addition of the reactants. A comparison with a chromatographic procedure reveled relative errors of -1.00 to - 5.26 %. The authors concluded that the method was relatively fast, easy to perform and can be a low cost alternative for pharmaceutical samples in which minoxidil concentration is relatively high. USP [11] method for assay and related substance for minoxidil in pure form and its formulations is by HPLC. BP[12] recommends potentiometric titration for assay of raw material. Thus, this method, although showed similar results when compared to a chromatographic technique, presents no analytical novelty. As can been seem in this subsection, titrimetric methods are cost-effective, require simple instrumentation, are quick and easy to perform. Indeed, due to its lack of selectivity, this technique is falling into disuse. For this reason, recent papers that employ titrimetry are rarely found in international journals. Consequently, several citations that employing titrimetry have been published in local journals [27].

## 2.4 Electroanalytical Methods

Electrochemical techniques provide high sensitivity, low detection limits, associated with the use of simple and inexpensive instrumentation[28]. Electrogravimetry, coulometry, conductometry, potentiometry, polarography, voltammetry and amperometry are electrochemical techniques that have been employed for drug analysis in dosage forms. However, voltammetric techniques are by far the most employed electrochemical techniques and the literature brings comprehensive reviews about their use for drug analysis in dosage forms[28]. The following will describe some examples of electroanalytical methods applied in pharmaceutical analysis.

Babaei, Afrasiabi and Babazadeh constructed a new chemically modified electrode based on multiwalled carbon nanotube/chitosan modified glassy carbon electrode (MWCNTs- CHT/GCE) for simultaneous determination of acetaminophen (ACT) and mefenamic acid (MEF) in aqueous buffered media. The measurements were carried out by application of differential pulse voltammetry (DPV), cyclic voltammetry (CV) and chronoamperometry (CA) methods. According to the authors, the analytical performance of DPV method has been evaluated for detection of ACT and MEF in human serum, human urine and a pharmaceutical preparation with satisfactory results. USP [11] recommends spectrophotometry for determination of ACT in pure form and HPLC for its determination in pharmaceutical dosage forms. BP[12] recommends HPLC for analysis of related substances of ACT, titration for assay of raw material and suppositories, spectrophotometry for assay of tablets, and HPLC for assay of capsules and oral suspension. For MEF, USP method for related substance and assay of raw material and drug formulations is by HPLC. BP recommends thin-layer chromatography for related substances analysis, titration for assay of raw material and HPLC for analysis of its dosage forms. Although combinations of MEF and ACT are frequently prescribed, a monograph for simultaneous analysis of these two drugs was not found. Therefore, the proposed method is relevant, also is simple, inexpensive, quick and sensitive (detections limits of 0.66 μmol L-1 and 0.46 μmol L-1 for MEF and ACT, respectively). Besides, the authors demonstrated the method’s applicability in human serum, human urine and drug samples. Thus, this method is an interesting alternative to described methods [29].

Campestrini and coworkers employed a bismuth-film electrode for use in to quantify sulfadiazine in pharmaceutical formulations. The bismuth film was deposited ex situ onto a glassy carbon substrate. Analysis of two sulfa drugs was carried out by differential-pulse voltammetry in 0.05 mol L−1 Britton–Robinson pH 4.5 solution. The authors concluded that the bismuth-film electrode was demonstrated to be suitable for direct cathodic voltammetric determination of sulfadiazine. USP [11] method for assay of this drug in pure form and its formulations is by HPLC. BP [12] recommends thin-layer chromatography for related substances analysis, and titration for assay of raw material and its dosage forms. The authors compared the precision of the proposed method to the BP standard titration method through statistical examination and they showed that the results are equivalent. The positives of this method are the simplicity, good sensitivity (detection limit of 2.1 μmol L-1), low-cost and possibility of application to pharmaceuticals [30].

Jain and coworkers studied electroreduction and adsorption of cefixime in phosphate buffer by cyclic voltammetry (CV), differential pulse cathodic adsorptive stripping voltammetry (DPCAdSV), and square-wave cathodic adsorptive stripping voltammetry (SWCAdSV) at hanging mercury drop electrode (HMDE). These voltammetric procedures were applied for the trace determination of the bulk drug in pharmaceutical formulations and in human urine using the follows experimental parameters: accumulation potential = -0.1 V (vs. Ag/AgCl, 3 MKCl), accumulation time = 50 s, frequency = 140 Hz, pulse amplitude = 0.07 V, and scan increment = 10 mV in phosphate buffer (pH 2.6). The authors concluded that the principal advantages of the proposed voltammetric method are its rapidity and simplicity. USP [11] and EP [18] method for assay of cefixime in raw material and its formulations is by HPLC. Also, EP describes a HPLC method for analysis of related substances of this drug. An interesting point is that the authors applied the method to determine cefixime in pharmaceutical formulation and spiked human urine. The positives of this method are the simplicity, rapidity, it involves no cleanup procedures and has a good sensitivity (detection limit of 3.99 ng mL-1)[31].

Lima-Neto and coworkers developed an electroanalytical procedure for determination of nitrofurantoin (NFT) and investigated the influence of the boron-doping levels in boron-doped diamond film electrodes on the electrochemical response of NFT. The investigations were carried out using the techniques of cyclic voltammetry and square wave voltammetry. The authors stated that the level of boron-doping in the diamond film electrodes influenced the electrochemical reduction of NFT. The appropriate cyclic voltammetric response of NFT was obtained with Britton–Robinson buffer at pH 4 and for diamond films doped with 10 000 and 20 000 mgL−1 of boron. The authors tested the applicability of the proposed procedure using a commercial pharmaceutical formulation of NFT and concluded that the method presents the advantage of eliminating mercury waste and minimizing the adsorptive problems related to the use of other electrodic solid surfaces. USP [11] method for assay of NFT in raw material and its formulations is by HPLC. BP[12] recommends thin-layer chromatography for related substances analysis and spectrofotometry for assay of raw material, oral suspension and tablets. The advantages of this method are that it did not require complex preparations or renovation of the electrode surface; it is environmentally friendly and sensitive (quantitation limit of 0.021 μg mL-1). However, the method was not applied to biological matrices, as seen in other reported electroanalytical methods [32].

Sartori and coworkers described the independent determination of propranolol (PROP) and atenolol (ATN) in pharmaceutical formulations using square-wave voltammetry and a cathodically pretreated boron doped diamond electrode. The electroanalytical determinations of propranolol or atenolol were carried out in 0.1 mol L−1 H2SO4 or 0.5 mol L−1 NaNO3 (pH 1.0, adjusted with concentrated $HNO_3$), respectively. According to the authors, the proposed method was successfully applied in the determination of both drugs in several pharmaceutical formulations (tablets), with results in close agreement at a 95% confidence level with those obtained using official spectrophotometric methods. USP [11] method for assay of PROP in raw material and its formulations is by HPLC. EP [18] recommends HPLC for analysis of related substances and tritation for assay of raw material. For ATN, EP describes a HPLC method for analysis of related substances and tritation for assay of raw material. BP [12] recommends HPLC for analysis of related substances, titration for assay of raw material, spectrophotometry for quantitation of ATN injection, HPLC for assay of oral solution and spectrophotometry for tablets. The presented methods are interesting alternatives to pharmacopoeial methods, because the simultaneous determination of these drugs is not described in USP, BP or EP. Additionally, the reported methods are sensitive (low detections limits for two drugs), simple, rapid, and dispenses any use of organic reagents or expensive apparatus. However, the authors do not consider an application of the method to biological matrices [33].

Veiga and coworkers used a multi-walled carbon nanotubes (MWCNTs) film-coated glassy carbon electrode (GCE) for the voltammetric determination of carbamazepine (CBZ). According to the authors, the voltammetric response of CBZ at this film-modified electrode increased significantly when compared with that at a bare glassy carbon electrode and the sensor response was reproducible. The proposed method was applied to the quantification of CBZ in wastewater samples, collected in a municipal wastewater treatment plant, and in pharmaceutical formulations. The authors stated that the developed methodology yields results in accord with those obtained by chromatographic techniques. USP [11] method for assay of CBZ in raw material and its formulations is by HPLC. BP [12] recommends HPLC method for analysis of related substances and for assay of raw material and tables. The method described by Veiga and coworkers is a relevant alternative to pharmacopoeial methods, because is simple, rapid, low cost and highly sensitive (limits of detection and quantification of 40 and 140 nMol L-1, respectively). Moreover, the authors applied the proposed method to the quantification of CBZ in commercially available medicinal tablets and in waste water samples and compared the method with those obtained by HPLC–UV and LC–MS techniques, showing that the results are in accordance. From these examples, it can be seen that electroanalytical methods are a good alternative to HPLC methods for assay of drugs formulations. The advantages of electrochemical techniques (mainly voltammetry) are good sensitivity, reasonable speed, minimal sample pre-treatment, satisfactory selectivity, wide applicability, and low cost of instrumentation and maintenance. In this subsection, we cited applications in environmental analysis and in biological matrices, which demonstrates the usefulness of this technique. However, much work is required before voltammetry can be adopted as a routine analytical method. Besides, this technique is not often used in analysis of related substances, as is observed with powerful separation techniques such as HPLC and capillary electrophoresis [34].

## 2.5 Chromatographic methods

### 2.5.1 Thin-layer chromatography

Thin-layer chromatography (TLC) is a reliable, simple and sensitive, rapid, and inexpensive technique that is applied for the analysis of pharmaceutical products. TLC has been shown in many publications to provide excellent separation and qualitative and quantitative analysis of a huge range of organic and metal-organic compounds [35] . In pharmaceutical analysis, TLC is predominantly used in its semiquantitative mode, where spots of reference tests solutions are visually against the impurity spots in a chromatogram of the test sample. However, most of the TLC procedures are considered an obsolete form of impurity testing. In addition, the progress in sorbent layers and instrumentation has led to an improvement of the reliability of quantitative planar chromatography, making this technique an economical alternative that competes with and complements HPLC [36] . Below are listed some recent examples of TLC applications in the field of pharmaceutical analysis.

Abdel-Fattah and coworkers developed three stability-indicating assay methods for the determination of tropisetron in a pharmaceutical dosage form in the presence of its degradation products. The proposed techniques were HPLC, TLC, and first-derivative spectrophotometry. The

authors carried out an acid degradation, separated the degradation products by TLC and identified by IR, NMR, and MS techniques. The TLC method was based on the separation of tropisetron and its acid-induced degradation products, followed by densitometric measurement of the intact spot at 285 nm. The separation was carried out on silica gel 60 F254 aluminum sheets using methanol–glacial acetic acid (22:3, v/v) mobile phase. According to the authors, the suggested methods were successfully applied for the determination of the drug in bulk powder, laboratory-prepared mixtures, and a commercial sample. BP [12] recommends HPLC for analysis of related substances of tropisetron and potentiometric titration for assay of raw material. TLC shows to be a good alternative to HPLC and spectrophotometric methods, as well as the methods proposed by BP because this method provided simple, accurate, and sensitive quantitative determination of tropisetron in pharmaceutical formulations. Moreover, the method was compared with a HPLC reported method and the results were statistically the same [37].

Kadukar and coworkers developed and validated a densitometric method for quantitative determination of olmesartan medoxomil (OM) and hydrochlorothiazide (HTZ) in combined tablet dosage forms. Separation of the drugs was carried out using chloroform–methanol–toluene 6:4:5 (v/v/v) as mobile phase on precoated silica gel 60 F254 plates. The detection of bands was carried out at 258 nm. According to the authors, the method can be used for routine analysis of these drugs in combined tablet dosage forms in quality control laboratories. As already mentioned, several analytical techniques are mentioned in pharmacopoeias for analysis of HTZ. However, no USP [11], BP [12], or EP [18] has been reported a method for the determination of OM and for simultaneous analysis of HTZ and OM. Therefore, the presented method, although simple, can be used in analytical laboratories that have limited resources [38].

Kakde, Satone, and Bawane developed a high-performance thin-layer chromatographic (HPTLC) method for simultaneous quantitative analysis of escitalopram oxalate (ESC) and clonazepam (CLO) in pharmaceutical preparations. Separation was achieved on a aluminum HPTLC plates coated with 0.2 mm layers of silica gel 60 F254 with methanol–toluene–triethylamine 1:3.5:0.1 (v/v) as mobile phase. Densitometric quantification was performed at 253 nm by reflectance scanning. The authors concluded that the method can be applied for routine quality-control analysis of ESC and CLO in pharmaceutical preparations and it is suitable for detection of degradation products of clonazepam in bulk and pharmaceutical preparations. USP [11] recommends HPLC for analysis of related substances, raw material, oral suspension and tablets of CLO. BP [12] describes HPLC for analysis of related substances, potentiometric titration for assay of raw material and spectrophotometry for assay of CLO injection. The method cited above seems to be a good alternative to reported methods, because it provides to be simple, rapid and sensitive for simultaneous analysis of both drugs and their degradation products in pharmaceutical preparations [39].

Maślanka, Krzek and Stolarczyk developed a chromatographic and densitometric method for identification and quantitative analysis of hydrochlorothiazide, triamterene, furosemide, and spironolactone in drugs used to treat hypertension. For separation, silica gel F254 plates were used with hexane–ethyl acetate–methanol–water–acetic acid 8.4:8:3:0.4:0.2 (v/v) as mobile phase. Densitometric measurements were performed at 264 nm. The authors stated that the method is suitable for use in analytical laboratories. Although several methods have been reported in pharmacopoeias for analysis of these drugs, a method for its simultaneous quantitative determination was not found in the official compendium surveyed [40].

Mehta and Morge reported a HPTLC method for quantitative analysis of candesartan cilexetil and hydrochlorothiazide in their tablet dosage forms. Chromatography was performed on silica gel 60 GF254 plates with acetone–chloroform– ethyl acetate–methanol 3:3:3:0.5 (v/v) as mobile phase. Detection was performed at 280 nm. This method has advantages because no pharmacopoeia monograph is described for simultaneous analysis of these active substances. Furthermore, the method was properly validated and shows satisfactory sensitivity [41].

Miracor and coworkers published an HPTLC method using an internal standard for analysis of colchicine in a pharmaceutical formulation. The analyte and internal standard were separated on aluminum plates precoated with silica gel 60 F254; the mobile phase was ethyl acetate– acetonitrile–water–formic acid 8.0:1.0:0.5:0.5 (v/v). Quantification was by densitometric scanning at 358 nm. According to the authors, the established method enabled accurate, precise, and rapid analysis of colchicine in the pharmaceutical formulation. BP [12] recommends HPLC method for analysis of related substances, potentiometric titration for assay of raw material and spectrophotometry for tablets. Although the cited method is a simple and cheap , no analytical advantage is demonstrated. From the revised studies that employ thin layer chromatography for pharmaceutical analysis, it was seen that advantages of this technique include fast analyses, low cost, and less organic solvent wastage [42].

**2.5.2 Gas chromatography**

Gas chromatography (GC) is a sensitive, accurate, reproducible, quantitative, and versatile tool and well adapted for the analysis of complex mixtures and this technique plays significant role in the analysis of drugs and pharmaceutical products. However, the use of CG is limited to volatile and thermally stable compounds or to molecules that may undergo derivatization reactions to form thermally stable products. Below are summarized some important applications of this technique in the field of pharmaceutical analysis.

Farajzadeh and coworkers reported a technique for the extraction of valproic acid (VPA) by hollow-fiber coated wire as a lab-made solidphase microextraction (SPME) fiber and its determination by capillary gas chromatography in human serum and pharmaceutical formulations. The authors used a piece of copper wire coated by polypropylene hollow-fiber membrane as a SPME fiber, and its efficiency for the extraction of VPA was evaluated. The microextraction process was optimized by the authors and the chromatographic conditions were set as follows: Initial column temperature maintained at 75°C for 1 min, and then raised at 20°C min-1 to

240°C and held for 5 min. Nitrogen was used as carried and make up gas with flow rates of 1.2 and 45 mL min-1, respectively. The temperatures of the inject port and the detector were set at 145 and 250°C, respectively. Injections were carried out in splitless mode. The authors described that the low detection limit, wide linear dynamic range, repeatability and higher mechanical durability due to its metallic base are some of the most important advantages of the this fiber. USP [11] recommends GC for analysis of VPA drug substance and for its formulations. BP [12] recommends GC for related substances and potentiometric titration for assay of drug substance. In comparison with pharmacopoeial methods, the microextraction procedure seems to be easy to perform. Moreover, the method was compared with a previously reported method and the authors demonstrated a considerably higher sensitivity. Also, the application of the proposed method for the monitoring VPA in human serum is very useful for clinical laboratories [43].

Yilmaz published a gas chromatography– mass spectrometry (GC-MS) method for simultaneous determination of estradiol valerate (EV) and medroxyprogesterone acetate (MPA) in a tablet formulation. Chromatographic analysis was carried out on an HP-5 MS column with 0.24 µm film thickness (30 m × 0.30 mm). Splitless injection was used and the carrier gas was helium at a flow rate of 2 mL min-1. The injector and detector temperatures were 25°C. The MS detector parameters were transfer line temperature 28°C, solvent delay 3 min and electron energy 75 eV. The oven temperature program was held at 15°C for 1.5 min, increased to 26°C at a rate of 50°C min-1 for 1 min and then increased to 275°C at a rate of 10°C min-1 for 3.5 min. The author concluded that themethod can be used for the routine QC analysis of simultaneous determination of EV and MPA pharmaceutical preparations in a total time of 15 min. For these two drugs, USP [11] recommends HPLC method for analysis of drug substance and its drug formulations. BP [12] method for related substances of EV is by HPLC and for assay of drug substance is by spectrophotometry. For MPA, BP [12] recommends HPLC method for related substances, spectrophotometry for assay of drug substance and HPLC for assay of drug formulations. Therefore, the advantage of this method is the application for simultaneous analysis of both drugs. Moreover, it shows to be sensitive (detection limit of 10 and 25 ng mL-1 for EV and MPA, respectively) and selective from impurities and excipients peaks. From these reviewed papers, it may be noted that although gas chromatography is an analytical method that serves a broad range of pharmaceuticals, this technique suffers from a major limitation in that a relatively small range of volatile and thermally stable compounds that are reliable for analysis [44].

### 2.5.3 High and ultra performance liquid chromatography

High performance liquid chromatography (HPLC) is the most popular technique used for the analysis of pharmaceuticals [45] and reversed-phase HPLC (RP-HPLC) is the most popular LC technique for pharmaceutical analysis [46]. Below are exemplified some recent application of HPLC in pharmaceutical counterfeit analysis.

Casagrande and coworkers developed and validated a HPLC method for the quantitative evaluation of quercetin in topical emulsions. Chromatography was performed on a HypersilR BDS-CPS C18 column (4 µm; 250 x 4.5 mm i.d.) with a mobile phase of methanol: water (35:65; v/v) containing 2% acetic acid and flow rate of 1 mL/min. The volume injected was 25 µL with detection at 254 nm. The authors evaluated the Physico-chemical stability and showed that the raw material andanionic emulsion, but not non-ionic emulsion, were stable in all storage conditions for six months. The authors concluded that the present work reports a fast and sensitive HPLC technique useful for routine quality control for quercetin determination in topical formulations. No USP [11], BP [12], or EP [18] has been reported a method for the determination of quercetin. Thus, this method is useful for routine quality control analysis because it was demonstrated a short analysis time (approximately 8.5 minutes) and a high sensitivity (18 and 29 ng mL-1 for detection and quantitation limits) [47].

Chopra and coworkers described a comparative study of different detectors for the liquid chromatographic determination of tobramycin. Due to the absence of a significant UV chromophores in the structure of tobramycin, the authors made a comparison between the analytical performance of different brands of pulsed electrochemical detectors (PED) and two different evaporative light scattering detectors (ELSD) from the same manufacturer. Different PED waveforms (triple, quadruple and six potential–time waveforms (TPW QPW and SPW, respectively)) were also examined for the determination of tobramycin. USP [11] recommends a HPLC method using a derivatization procedure for analysis of tobramycin. BP [12] and EP [18] prescribe a LC–PED method for assay of drug substance and a microbiological test for assay of tobramycin injection.. This paper has a contribution to literature, because it shows alternative detection tools to derivatization procedure proposed by USP and it compares the sensitivity of the considered detectors, showing that PED with SPW has the best analytical performance [48].

D'Hondt and coworkers published and validated an HPLC method for the assay and degradation of salmon calcitonin at low concentrations (400 ppm) in a bioadhesive nasal powder containing polymers. The authors used an HPLC-UV/MS method with a reversed-phase C18 column in a gradient system based on aqueous acid and acetonitrile. UV detection, using trifluoroacetic acid in the mobile phase, was applied for the assay of calcitonin and related degradants. Electrospray ionization (ESI) ion trap mass spectrometry, using formic acid in the mobile phase, was implemented for the satisfactory identification of degradation products. The authors states that for the first time, a complete quality control of salmon calcitonin in a solid pharmaceutical powder was performed. BP[12] and EP[18] recommend HPLC method for analysis of related substance and for assay of raw material. However, a method for analysis of this drug in bioadhesive nasal powder was not found in the reviewed pharmacopoeias. Hence, this method is useful to control quality laboratories. Moreover, the authors demonstrated a satisfactory sensitivity for degradations products of calcitonin (detection limits ranging between 0.5 and 2.4%, compared to main calcitonin peak) [49].

Davydova and coworkers reported a dissolution test method and an analytical procedure by HPLC for evaluation of the dissolution characteristics of dietary supplements tablets containing vitamin A. Seven differen products containing retinyl acetate or retinyl palmitate were analysed. Chromatographic analysis was carried out on a Waters Alliance HPLC system using Zorbax $NH_2$, 15 cm ×4.6 mm, 5 μm column. Hexane was used as mobile phase with a flow rate of 2 mL min-1. Injection volume was 42 microliters with UV detection at 326 nm. EP [18], BP [12], and USP [11] have general chapters for dissolution, but these pharmacopeias do not have monographs with specifications for vitamin A in tablets. Therefore, the dissolution test described could be proposed as a pharmacopoeial standard to assess the performance of tablet formulations containing vitamin A. Moreover, the authors demonstrated a satisfactory analysis time (14 min) [50].

Mone and Chandrasekhar published degradation profile of pentoxifylline, which was subjected to various stress conditions. A stability indicating LC separation method was developed for pentoxifylline and its three degradation products using 1.8 μm, C18 reverse phase column and UPLC. According to the authors, baseline separation was done with a run time of 5 min. Under oxidative stress conditions the drug substance underwent distinct transformation to give rise to a single major degradation product which was elucidated using 1D, 2D NMR spectroscopy and high resolution mass spectrometry (Q-TOF LC/MS). The authors concluded that in this report, it was successfully used sub 2 μ reverse phase column technology, with UPLC system for the development of efficient and robust method for the separation of closely related compounds of pentoxifylline. USP[11] recommends HPLC for analysis of pentoxifylline drug substance and its formulations. BP[12] and EP[18] describe HPLC technique for related substances and potentiometric titration for assay of active drug. However, the novelty of this method was the elucidation of a new degradation product of this drug. The detections and quantitation limits for this degradation product were found to be 0.4 and 2 μg mL−1, respectively, showing attractive and effective method sensitivity. Moreover, although UPLC technique requires expensive instrumentation, it is considerably faster and generates less waste, when compared with HPLC techniques [51].

Rao and coworkers reported forced degradation of ritonavir (RTV) under the conditions of hydrolysis (acidic, basic and neutral), oxidation, photolysis and thermal stress using a LC–MS/MS method. Eight degradation products were formed and their separation was done on Waters XTerraR C18 column (252 mm× 4.6mm, 5 μm) using water :methanol:acetonitrile (40:20:40, v/v/v) as mobile phase. According to the authors, no previous reports were observed in the literature regarding the characterization of degradation products of RTV. USP [11], BP [12] and EP [18] recommend HPLC for analysis of related substances and for assay of drug substance. The importance of the proposed method is that the authors extended the method to LC–MS/MS for characterization of the degradation products. Moreover, in comparison with the pharmacopoeial methods, the chromatographic running time of this technique was significantly lower. The limits of detection and quantification were found to be 10 and 30 ng mL-1, respectively,showing good method sensitivity [52].

Zanitti and coworkers reported a semipreparative HPLC enantioseparation of omeprazole (OME) and its potential organic chiral impurities on the immobilised-type Chiralpak IA chiral stationary phase (CSP) under both polar organic and normalphase condition. The (S) enantiomers were isolated with a purity of >97% and their absolute configuration were empirically assigned by circular dichroism (CD) spectroscopy. The technique used a mixture methyl tert-butylether (MtBE)–ethyl acetate (EA)–ethanol (EtOH)–diethylamine (DEA) 60:40:5:0.1 (v/v/v/v) as a mobile phase. The authors described that the proposed method ensures the EP requirements for the routine check of enantiomeric purity in raw material and pharmaceutical preparations. Although USP[11], BP [12] and EP [18] already describe HPLC methods for analysis of OMP, the importance of this work is a semipreparative enantioseparation of this drug and its chiral related substances. The detection and quantitation limits were estimated to be 168 and 514 ng mL-1 for (S)- OME enantiomer, showing the methodsensitivity.The papers cited in this subsection demonstrate the applicability of this automated technique, which allows analysis of related substances, elucidation of degradation products, stability studies, quantitation of samples from dissolution tests, chiral separations, determination of drugs in biological matrices, and a multitude of other applications. Moreover, the large varieties of detections systems and chromatographic columns available make possible the analysis of virtually all pharmaceutical compounds. However, there is a high cost for equipment required to conduct HPLC and this technique can generate huge amount of waste. The problem of waste disposal, however, is being reduced with the use of chromatographic columns packed with smaller particles, resulting in significantly faster analyses with consequent reduction in generation of waste [53].

**2.6 Capillary electrophoresis**

Capillary electrophoresis is an effective separation technique that can be used to large and small molecules. This technique has high efficiency of separation enabling difficult separations, low analysis duration, fast development of methods, low sample and solvent consumption, and automated and simple instrumentation[54]. The instrumentation of CE consists of electrodes, a sample introduction system, a capillary, a high voltage power supply, a detector, and a data output and handling device[1]. There are many techniques that can to help separate analytes with a capillary electrophoresis system. These include non-aqueous CE (NACE), microemulsion electrokinetic chromatography (MEEKC), capillary isotachophoresis (CITP), capillary electro chromatography (CEC) and immunoaffinity capillary electrophoresis (IACE). Moreover, different means of detection (MS, lightemitting diode, fluorescence, chemiluminescence and contactless conductivity (C4D) detectors) are used, giving versatility and sensitivity to CE[1]. The following are some applications of this technique for pharmaceutical counterfeit analysis.

Sanchez-Hernandez and coworkers published a capillary electrophoresis–electrospray ionization-tandem mass spectrometry to the quantification of l- and d-carnitine in pharmaceutical formulations. A sample treatment method consisting of the use of a dilution or an extraction step with water was employed prior to derivatization with 9-fluorenylmethoxycarbonyl (FMOC). More than ten pharmaceutical formulations were analyzed including ampoules, oral solutions, sachets, and tablets. Results shows contents for carnitine comprised between 76 and 100% with respect to the labeled ones in the case of those formulations marketed with the racemate, and from 97 to 102% in those cases where the single enantiomer (l-carnitine) was employed as active ingredient. USP [11] describes titration with perchloric acid for assay of l-carnitine raw material, and HPLC for assay of its drug formulations. BP [12] and EP [18] describe an HPLC method for determination of related substances and titration with perchloric acid for assay of l-carnitine raw material. However, only an optical purity control of lcarnitine as raw material is required, which lacks of sensitivity. Hence, there is a potential of the proposed method to be applied in quality control containing carnitine. Moreover, the detection limit of 11 mg mL−1 (0.002%) in drug product was considerably lower than the limit established for any impurity (0.1%), showing the method sensitivity [55].

Azzam, Saad and Aboul-Enein proposed a capillary zone electrophoresis method for the combined determination of atenolol, chlorthalidone and amiloride in pharmaceutical formulations in less than 3.5 min. The separation is effected in an uncoated fused-silica capillary (74 μm i.d. × 52 cm) and a background electrolyte of 25 mm $H_3PO_4$ adjusted with 1 mol L-1 NaOH solution (pH 9.0) and detection at 197 nm. Normal polarity mode at 24°C, 25 kV and hydrodynamic injection (10 s) were applied. According to the authors, the method was applied to the simultaneous determination of tested drugs in various pharmaceutical tablets formulations. USP[11], BP [12] and EP[18] describe methods for individual analysis of AT, CD and AM as HPLC, TLC, titration and spectrophotometry. However, a method for the simultaneous determination of these drugs has not been described by the abovementioned pharmacopoeias. Therefore, the reported method is useful and can be adopted as a quality control in pharmaceutical counterfeit estimation. Moreover, the method shows a short run time for separation of the drugs (about 3.5 min) and adequate sensitivity (the limits of quantitation for AT, CD and AM were 2.35, 1.43 and 4.96 μg mL-1, respectively [56].

Kika and coworkers reported a capillary zone electrophoretic (CZE) technique for the determination of mitoxantrone (MTX) in pharmaceutical formulations. Separation was achieved with 24mM ammonium acetate at an apparent pH value of 5.0 in 50% v/v acetonitrile, applied voltage of +29 kV, and capillary temperature of 24°C. Detection was carried out at 242 nm. Mitoxantrone and doxorubicin (DOX) were completely separated in less than 7 min. This method was applied for the analysis of MTX in its injectable pharmaceutical formulation.

According to the USP[11], BP[12] and EP [18], an ion-pair HPLC method is recommended for the quality control of the MTX drug substance and in injectable form. However, the disadvantage of ion pairing chromatography are prolonged equilibration times, difficult regeneration of the column, and irreproducible retention times. Hence, this technique is an effective way to pharmacopoeial method. However, the method provided adequate, although low sensitivity (detection limit of 5 µg mL-1) [57].

Rambla-Alegre and coworkers published a capillary electrophoresis (CE) procedure combined with UV detection for the quantitative determination of ethylendiamine, ethanolamine, propylamine, piperazine and other derivative groups in serum samples as well as in pharmaceuticals. The optimized parameters for the quantification of antihistamines were a 23 cm capillary, UV detection at 215 nm, 20 mMol L-1 phosphate running buffer at pH 2.0, 2 psi s−1 injection pressure and 5 kV applied voltage. Under these conditions, the analysis time was below 10 min. This is an effective work from an analytical point of view because no USP [11], BP [12] or EP [18] describes a method for simultaneous analysis of these drugs. Also, the method shows a high sensitivity for all analyzed compounds (detections limits ranging from 4 to 28 pg mL−1) and it was suitable for the determination of antihistamines [58].

Zhu and coworkers reported the separation of the sibutramine enantiomers by capillary zone electrophoresis using substituted cyclodextrins as chiral selectors. Separation of enantiomers on an unmodified fused silica capillary (effective length 44 cm) was achieved using a mixed buffer of 20 mMol L-1 phosphate and 10 mMol L-1 citrate containing either 5 mMol L-1 methyl-β-cyclodextrin (pH 4.3) or 5 mMol L-1 carboxymethyl-β-cyclodextrin (pH 6.5). Samples were injected with a pressure of 50 mbar for 5 s and were detected at a wavelength of 222 nm. No USP [11], BP [12] or EP [18] describes a monograph for sibutramine. From the papers cited in this subsection, it was concluded that capillary electrophoresis offers significant advantages like small sample volumes, fast analysis, high separation efficiency, low consumption of reagents and low wastage. However, this method frequently has low sensitivity, due to the shorter path length of the flow cell [59].

### 2.7 Vibrational spectroscopies

Vibrational chemical analysis strategies carries with it three main tools: mid IR spectro metry, additional usuallynamed Fourier rework IR (FTIR) spectrometry, near-IR (NIR) spectrometry, and Raman spectrometry. Some applications of this method in pharmaceutical analysis are summarized below.

Li and coworkers developed away supported Fourier remodel close to infrared(FT-NIR)spectroscopy and partial statistical procedure (PLS) algorithmic program for measure of 4 sorts of Tanreqing injection inter mediates. The Near infrared spectra of a hundred and twenty samples were collected in transflective mode. The concentrations of chlorogenic acid, caffeic acid, luteoloside, baicalin, ursodesoxycholic acid (UDCA), and chenodeoxycholic acid (CDCA) were determined with the HPLC–DAD/ELSD as reference technique. The established models were used for the liquid preparation method analysis of tanreqing injection in 3 batches. As no pharmacopoeial technique is delineated for the analyzed compounds, this can be a relevant work. Moreover, the strategy shows satisfactory sensitivity (detections limits starting from zero.285 to 95.8 ng). The most advantage incontestible during this work is that the planned technique is speedy and non destructive to get the idea for the intermediates of Tanreqing injection[60].

Oh and coworkers published a method for detection of TiO2 concentration in a cream formulation using Raman spectroscopy without further sample pretreatments. According to the authors, the distribution of TiO2 particles in a highly viscous cream may not be homogeneous on a microscopic scale and local aggregation of the particles is possible; therefore, acquisition of Raman spectra capable of representing the whole sample identity. A wide area illumination (WAI) scheme, applying 6 mm diameter laser illumination area on a sample, was used to obtain representative sample presentation and improved accuracy. BP[12] and EP[18] recommend titration with 0.1 M ammonium and cerium nitrate for analysis of TiO2. The proposed method would be advantageous over the conventional titration method that requires destructive incineration of the organic cream matrix, as well as a higher consumption of chemical [61].

Talebpour and coworkers presented a technique for the simultaneous estimation of penicillin G salts in pharmaceutical mixture via FTIR spectroscopy combined with chemometrics. The authors predict that the mixture of penicillin G salts is a complex system due to similar analytical characteristics of components. Partial least squares (PLS) and radial basis function-partial least squares (RBF-PLS) were used to develop the linear and nonlinear relation between spectra and components, respectively. The orthogonal signal correction preprocessing method was used to correct unexpected information, such as spectral overlapping and scattering effects. The chemometric models were tested on an outer dataset and applied to the detection of commercialized injection product of penicillin G salts. Because no pharmacopoeia describes a monograph for simultaneous analysis of studied penicillin G salts (potassium penicillin G, benzathine penicillin G, and procaine penicillin G), this is an interesting approach. However, the sensitivity of the method is not presented[62].

Hu and coworkers demonstrate the combined quantitative analysis of sulfathiazole polymorphs (forms I, III and V) in ternary mixtures by attenuated total reflectance-infrared (ATR-IR), near-infrared and Raman spectroscopy combined with multivariate analysis. To minimize the effect of systematic variations, four different data pre-processing methods; multiplicative scatter correction (MSC), standard normal variate (SNV), first and second derivatives were employed and their performance was evaluated using their prediction errors. According to the authors, it was possible to derive a reliable calibration model for the three polymorphic forms, in powder ternary mixtures, using a partial least squares algorithm with SNV pre-processing, which predicted the concentration of polymorphs I, III and V. The USP [11] method for assay of sulfathiazole drug substance is titration with 0.1M sodium nitrite. BP[12] and EP[18] recommend TLC for related substances and titration with 0.1M sodium nitrite for assay of drug compounds. However, these pharmacopoeial methods are not able to quantify different polymorphic phases. Since polymorphs of a pharmaceutical solid may have different physicochemical properties, this work is quite relevant. The detection and quantitation limits for the Near infrared analyses were 3.6 and 10.8% for form I, 5.8 and 17.6% for form III and 6.3 and 18.5% for form V. According to the authors, these results are in the same limit as those reported for other ternary polymorph analyses [63].

Ziemons and coworkers published a near infrared calibration model to detect the acetaminophen content of a low-dose syrup formulation (2-3%, w/v). A prediction model was built using partial least square regression. First derivative followed by standard normal variate were chosen as signal preprocessing. The Near infrared model was used to monitor in real time the API concentration while mixing syrups containing various amounts of API and, according to the authors, a good relation was found between the Near infrared method and the theoretical concentrations. For acetaminophen, several pharmacopoeial techniques are described including High performance liquid chromatography, titration and spectrophotometry. However, the advantage of the method proposed by Ziemons and coworkers is that the Near infrared measurements could be performed off-line, at-line, on-line or in-line to check the conformity of the pharmaceutical syrups during the manufacturing and/or before the final packaging stage. Furthermore, the method presented a high, but adequate sensitivity (lower quantitation limit of 15.7 mg mL-1) From the above-mentioned vibrational spectroscopy methods, it was noted that the usefulness of this technique is mainly attributed to its speed and economy against other analytical techniques. Additionally, it offers the possibility to perform on-line analysis. The analytical targets are mainly powdered solids and the modes of NIR measurement are mainly diffused reflection[64].

## 3. CONCLUSION

In this paper, recent analytical methods applied for quantitative analysis of pharmaceutical formulations were reviewed. Several tools like UV/VIS spectrophotometry, fluorimetry, titrimetry, electroanalytical techniques (mainly voltammetry), chromatographic methods (TLC, CG and mainly HPLC), CE, and vibrational spectroscopies are the main techniques that have been used, of which it is found a tradition to utilize faster techniques with cost savings and reduction in solvent consumption. Table 1 presents a summary of cited techniques for the quantitative analysis of counterfeit pharmaceuticals drugs. From this work, it was observed a trend in the application of techniques increasingly rapid such as ultra performance liquid chromatography and the use of sensitive and specific detectors as mass spectrometers.

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**Table 1.** Examples of quantitative methods applied in pharmaceutical counterfeit analysis. The citations are grouped according to analytical techniques exemplified in this work.

| **Compound(s)** | **Sample(s)** | **Analytical technique(s)** | **Ref.** |
|---|---|---|---|
| Sildenafil citrate | Tablets | Raman Spectroscopy | 5 |
| Tadalafil | Tablets | NMR and Raman spectroscopy | 6 |
| Sildenafil, Tadalafil, Verdanafil | Tablets | NIR, RAMAN and FTIR | 7 |
| Atorvastatin | Tablets | NIR and RAMAN with chemometrics | 8 |
| Drotaverine hydrochloride, caffeine, paracetamol, | Tablets | UV spectrophotometric and HPLC | 9 |
| Fluphenazine, pernazine, haloperidol | Tablets | Spectrophotometry | 15 |
| Chlorzoxazone (CLZ) and ibuprofen | Capsules and human plasma | Fluorimetry | 16 |
| Rosiglitazone maleate | Bulk, Tablets and human plasma | Spectrofluorimetry and spectrophotometry | 17 |
| Verapamil hydrochloride, diltiazem hydrochloride, nicardipine hydrochloride and flunarizine | Tablets | Spectrofluorimetry | 19 |
| Camptothecin in Second derivative irinotecan and in opotecan | Tablets and capsules | Spectrofluorimeter | 20 |
| Hydrochlorothiazide, indapamide and xipamide | Tablets | Spectrofluorimeter and spectrophotometer | 21 |
| Doxepin hydrochloride | Capsules | Spectrofluorimeter | 22 |
| Stavudine | Tablets | Titrimetry and spectrophotometry | 23 |
| Nordiazepam | Tablets | Titrimetry and spectrophotometry | 24 |
| Hydroxyzine Dihydrochloride | Pure form and Tablets | Titrimetry | 25 |
| Doxycycline hyclate | Pure form and Tablets | Titrimetry and spectrophotometry | 26 |
| Minoxidil | Topical formulation | Titrimetry | 27 |
| Acetaminophen and mefenamic acid | Human serum and urine | Voltammetry and Amperometry | 29 |
| Sulfadiazine | Tablets | Voltammetry | 30 |
| Cefixime | Tablets and human urine | Votammetry | 31 |
| Nitrofurantoin | Capsules | Voltammetry | 32 |
| Propranolol and Atenolol | Tablets | Voltammetry | 33 |
| Carbamazepine | Tablets and waste water samples | Voltammetry | 34 |

| | | | |
|---|---|---|---|
| Tropisetron | Bulk powder,Laboratory prepared mixtures and capsules | HPLC,TLC and first derivative spectrophotometry | 37 |
| Olmesartan medoxomil And Hydrochlorothiazide | Tablets | TLC | 38 |
| Escitalopram oxalate | Bulk and Tablets | HPLC | 39 |
| Hydrochlorothiazide, triamterene, furosemide, and spironolactone | Tablets | TLC | 40 |
| Candesartan cilexetil and hydrochlorothiazide | Tablets | HPLC,TLC | 41 |
| Colchicine<br>Tablets<br>HPLC,TLC<br>42 | Tablets | HPLC,TLC | 42 |
| Valproic acid | Gas chromatography | Human serum, Tablets and syrup | 43 |
| Estradiol valerate and Medroxyprogesterone Acetate | Tablets | Gas chromatography and Mass spectrometry | 44 |
| Quercetin | Topical emulsions | HPLC | 47 |
| Tobramycin | Pure form | HPLC | 48 |
| Salmon calcitonin | Bioadhesive nasal power | HPLC | 49 |
| Retinyl acetate and retinyl palmitate | Tablets | HPLC | 50 |
| Pentoxifylline | Tablets | UPLC, LC-MS | 51 |
| Ritonavir | Pure form | LC-MS | 52 |
| Omeprazole | Raw materials and Tablets | HPLC | 53 |
| l-carnitine and dcarnitine | Ampoules, oral solutions and tablets | CE-MS | 55 |
| Atenolol, chlorthalidone and amiloride | Tablets | CZE | 56 |
| Mitoxantrone | Injectables | CZE | 57 |
| Ethylendiamine, ethanolamine | Serum sample, capsule and syrup | CE | 58 |
| Sibutramine | Capsules | CE | 59 |
| Chlorogenic acid, Caffeic acid, luteoloside, baicalin, ursodesoxycholic acid and chenodeoxycholiacid | Injectables | HPLC, FT-IR PDA | 60 |
| TiO2 | Cream formulations | Raman spectroscopy | 61 |
| Penicillin G salts | Pharmaceutical mixture | FT-IR | 62 |
| Sulfathiazole Polymorphs | Pharmaceutical mixture | FT-IR, RAMAN | 63 |
| Acetaminophen | | Near Infrared spectroscopy | 64 |

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