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LIMITATIONS AND REMEDIAL APPROACHESON ANALYTICAL METHODS FOR SELECTED CLASSES OF NON-CHROMOPHORE PHARMACEUTICALS - A SYSTEMATIC REVIEW

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ABSTRACT

The revolution in analytical method development of active pharmaceutical ingredients (APIs) in drug formulation can satisfy strict regulatory regulation to improve public health safety and quality assessment. Chemically, pharmaceuticals product possesses single or more APIs that have different functional groups, leading to diversified and unique physio-chemical properties. Among all functional groups, chromophore structure plays major role in some of the typical analytical method development and validation process. Chromophore usually denotes functional group that exhibits absorption of electromagnetic radiation in Ultraviolet-visible (UV-Vis) region. Chromophore functional groups consist of conjugated pi-bonding system including ethylene, acetylene, carbonyls, acids, esters and nitrile groups, which actively estimated with various spectroscopic techniques like UV-Visible spectrophotometry. However, if the molecule does not contain a chromophore group, it cannot be absorbed or is poorly absorbed under UVregion (190-380nm). This would render UV spectrophotometric method utilizing UV detector or fluorescence detector more difficult to detect these non-chromophore drugs for direct measurement. Sometimes, the process might need expensive chemical modifications through derivatization brings further complications in the analytical process and might affect important parameters including precision, accuracy and reproducibility of an analytical method. In this review, it is highlighted the role of different analytical instrumentation used in assessing the quality of drugs, such as immunoassays, spectrophotometric, chromatographic, electrophoretic, and electrochemical methods that have been applied in modern pharmaceutical analysis for non-chromophore drugs. The limitations of previously reported methods for the selected pharmaceutical classesas poor or non-chromophore molecules were also summarized. Few recommendations were also suggested to choose the right analytical method for a right molecule. This review also included future considerations to limit the usage of toxic solvents in the analytical method development and validation to make less complex, eco-friendly, timesaving, and yet cost-effective approach for new drug approval and regulatory requirements in pharmaceutical industries. This would meet the requirement of sustained development goals by United Nation (UN).

INTRODUCTION

Modern pharmaceutical analysis has been widely applied on the analytical investigation of bulk-drug materials, active pharmaceutical ingredients (APIs), drug intermediates during synthesis, excipients in drug formulation, drug products and its possible impurities. Dissolution testing of pharmaceuticals is also performed to correlate with the drug bioavailability and the efficacy of drug therapy, where sample solutions are drawn at frequent intervals in a dissolution medium to study drug release profiles during formulation research and development and finished drug products. These sample solutions are further analyzed using suitable analytical method (UVVisibleSpectroscopy and HPLC) to estimate the drug concentration at different time intervals. Drug product analysis under various stress conditions has also been paramount importance in determining the possible degradation process of a product that may occur during storage or transportation process. Therefore, the main objective of pharmaceutical analysis is to obtain data that can contribute to the safety of drug therapy with maximal clinical efficacy, and minimal cost during the production of drugs [1]. The efficacy, safety and cost of drug therapy are extremely important issues in view of public health, which dictates the financial power of any country. There is a need for research to establish affordable sophisticated analytical methods which can rapidly evaluate qualities of drug samples in large quantity. To fulfill the rapidly increasing demands in optimization of analytical measurements for pharmaceutical and biomedical analysis, great efforts have been made by many researchers for further development of analytical chemistry, through publications of massive number of books and articles focusing on this topic [2]. With the aid of guidelines set by authorities like US Food and Drug Authority (FDA) and International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), well-developed analytical tests with suitable methodology and instrument can properly determine the quality of a drug formulation.Various separation methods such as thin layer chromatography (TLC), Gas chromatography (GC), high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE) are often used in pharmaceutical industries for the evaluation of drug samples. Hyphenated methods are also of recent trend such as HPLC coupled with mass spectrometry (LC-MS), HPLC/MS(MS) or LC/MS(MS) have become the predominant method in the drug metabolism studies (both *in vitro* and *in vivo*), high-throughput analysis of drugs and metabolites, analysis and identification of impurities and degradation products in pharmaceuticals, and analysis of chiral impurities [3]. This is due to its high sensitivity and selectivity. Spectrophotometric methods are commonly used by many due to high availability and affordability of the instrumentation in small scale to large scale industries in developing countries, because of its simplicity of analytical procedure, selectivity, specificity, speed, good precision and accuracy. They are more economic as compared to chromatography and electrophoresis methods. Infrared (IR) and near-infrared (NIR) spectroscopy are mainly applied in the identification of drugs. IR has been replaced the usage of most classical color tests, while increased utilization of NIR for in-process control in manufacturing pharmaceutical formulations should be taken note of. Combination with chemometric techniques, mainly principal component analysis (PCA) and partial least squares (PLS) algorithms, could be used as a fast computational and analytical tool for identification of potential candidate drugs. IR and Raman spectroscopy, together with solid-phase nuclear magnetic resonance (NMR), X-ray diffraction and thermal methods are the up-to-date methods in solid-phase characterization, providing great aid in developing pharmaceutical formulations with optimal bioavailability [2,3,4,5].In recent decade, green chemistry has received great interest in developing chemical innovation to meet environmental and economic goals simultaneously. Green Chemistry has a framework of a cohesive set of Twelve Principles, applying to all aspects of the process life cycle from the raw materials used to the efficiency and safety of the transformation, the toxicity and

biodegradability of products and reagents used. The main aim of green chemistry is to reduce the production of hazard at all stages that may cause adverse consequences to human health and the environment. There are few points that will be heavily discussed in this review, including prevention of generating waste, methodology design that does not produce hazardous substances, prevention of unnecessary use of solvent or auxiliaries, and reduce use of derivatization process. UV-Visible spectroscopy is a favourite tool for routine analysis to perform for multicomponent formulations, biotherapeutic or complex matrix samples. This technique utilises the basic principle of electron excitation from lower to higher energy levels due to the absorption of visible (380-740nm) and UV radiation (190-380 nm). Beer-Lambert's Law states that absorbance of solution is directly proportional to the concentration of absorbing sample in the solution and the path length. Therefore, for a fixed path length, UV/Vis spectroscopy can determine the concentration of the sample in the solution from the light absorbed, indicating precise amount of energy that causes transition of energy level. UV-Vis spectrophotometric methods were developed based on principle of additivity and absorbance, recording and mathematical processing on absorption spectra of standard and sample solutions in same way or differently. Since most analytes of interest are absorbing in the same spectral region as other compounds in the drug formulation, classical UV method could not accurately determine the concentration. Hence, development of different UV spectroscopic analytical techniques can be used in different scenario according to their nature. For example, simultaneous and derivative spectroscopy can be used to analyse both binary and tertiary mixture, where derivatives spectroscopy is advantageous for resolving closely absorbing peaks while simultaneous spectroscopy may be preferred for its simplicity. Variants based on derivative spectroscopy like ratio derivative spectroscopy, successive ratio derivative spectroscopy is better in terms of eliminating chemical interferences [6,7,8,9].

Due to lack of chromophore group, not all drugs are suitable to develop UV method. Some methods developed have used expensive reagents and time-consuming to conduct complex derivatization for UV detection. In this paper, we will briefly review the analytical methods in use for non-chromophore drugs, mainly aminoglycosides, bisphosphonate, gabapentin and pregabalin anticonvulsant. The challenges in the analytical method development and ways to overcome the limitation are addressed as well.

Aminoglycosides

Aminoglycosides (AGs) are broad-spectrum bactericidal agents potent against some Gram-positive and most Gram-negative bacterial bacillary infections. Even though AGs possess major adverse effects such as ototoxicity and nephrotoxicity, and with the introduction of newer and less toxic antimicrobials, AGs are still favorably used in various applications due to its low cost. The analysis of AGs and their related products is important in drug formulations and in therapeutic drug monitoring (TDM) in body fluids and tissues. In addition, analysis of AGs is important for veterinary applications and in environmental samples (water and soil). Aminoglycosides are characterized by two or more amino sugars as core structure, which is connected via glycosidic linkages to a dibasic aminocyclitol, which is most commonly 2-deoxystreptamine group. Aminoglycosides are broadly classified into four subclasses based on the identity of the aminocyclitol moiety: (*1*) no deoxystreptamine (e.g., streptomycin, which has a streptidine ring); (*2*) a mono-substituted deoxystreptamine ring (e.g., apramycin); (*3*) a 4,5-disubstituted deoxystreptamine ring (e.g., neomycin, ribostamycin); or (*4*) a 4,6-di-substituted deoxystreptamine ring (e.g., gentamicin, amikacin, tobramycin, sisomicin and plazomicin). The structures of significant AGs are shown in Figure 1 above. The amino sugar is decorated with a variety of amino and hydroxyl substitutions which play important role in mechanisms of action of AGs and their susceptibility to aminoglycoside-modifying enzymes [10,11]. Qualitative methods for aminoglycoside analysis include X-ray crystallography, NMR and MS method, while quantitative determination of aminoglycosides can be done with microbiological assay, various immunoassays, spectrophotometric, gas

chromatography (GC), thin-layer chromatography (TLC), HPLC, and capillary electrophoresis (CE). Different application would require suitable analytical method to serve its purpose. For example, microbiological assays are useful for semi-quantitative screening tests for the analysis of veterinary drug residues in food, but rapid enzyme immunoassays can accurately measure the concentration of AGs in complex matrices. Automated immunoassays are the most appropriate methods for AGs determinations in serum samples during TDM, while HPLC techniques provide good specificity and sensitivity required for pharmacokinetic and other research studies [12]. AGs are very hydrophilic compounds present in poly-ionic form in aqueous solution, its poor retention in reversed-phase LC column has made extraction and separation process difficult to achieve. The use of ion-pair liquid chromatography (IPLC) or HILIC seems to be the most straightforward ways to solve this problem. HILIC is more readily combined with electrospray ionization-MS (ESI-MS) detection than IPLC. Moreover, recent advancement in the detectors including pulsed amperometry detectors (PAD), ELSD and MS/MS can improve their determination to some extent. In addition to this, more advanced non-chromatographic methods have been reported in which gold nanoparticles (AuNPs) colorimetric method is used for the detection of kanamycin A and streptomycin in milk [13,14].However, these advanced techniques are not applicable in developing countries with lower economic power as these instruments are too expensive to afford, and the technique require highly trained personnel to conduct. The total number of LC–MS/MS instruments is limited in these countries and their use is mostly restricted to cost-worthy bioequivalence studies. The LC–PAD is even rarer in these countries, but much less expensive than LC–MS/MS. Also, detection using PAD requires post-column addition of NaOH to increase pH to 12 [12,15,16].

Assay of bulk pharmaceuticals and their formulation may be adequate with the use of simple spectrophotometric methods like UV/Vis spectrophotometry or infrared analysis. This type of fast and relatively simple tests can only be applied to not-too-complex matrices. However, the analysis of AGs is hindered by the lack of chromophore functional group and make direct UV detection unfeasible unless at a wavelength of 195 nm, which is not applicable in complex matrices. AGs can be subjected to pre- or post-column derivatization to introduce a chromophore, thus enabling UV detection. The main drawbacks of conducting pre- or post-column derivatization are long time consumption, labor intensive, and large overall variability due to extra sample preparation steps. Thus, more direct method such as infrared and Raman spectroscopy, which do not require derivatization or addition of solvent should be studied further.

Bisphosphonate Drugs

Bisphosphonate is a class of drugs which that inhibit osteoclast action and the resorption of bone. They are generally used to treat a variety of bone diseases such as hypercalcemia of malignancy, Paget's diseaseand osteoporosis. In general, bisphosphonates can be categorized as non-N-containing bisphosphonate (etidronate, clodronate and tiludronate) and N-containing bisphosphonate (pamidronate,neridronate, olpadronate, alendronate, ibandronate, risedronate and zoledronate).Separation analytical methods dedicated to the analysis of bisphosphonates have been previously reviewed by Sparidans and den Hartigh in 1999, then by Zacharis and Tzanavaras in 2008. [40,41] According to Sparidans and den Hartigh's review, initial application of radiolabeling on bisphosphonate drugs can provide sample quantification method, however it is not acceptable for the determination in biological samples of human origin. They have also summarized the chemical nature of bisphosphonates that causes several analytical difficulties, including strongly polar and ionic property that makes it hard to retain on non-polar stationary phase such as C18 or C8 column, complexation with metal ions and other cations which may produce chromatographic peak tailing,non-volatile property which cannot be analyzed easily with GC, and lack of suitable functional group for UV or fluorescence detection due to structural simplicity. From Figure 2, alendronate, pamidronate, neridronate, olpadronate,

ibandronate, etidronate and clodronate do not contain a chromophoric functional group. Development of a chromatographic assay for this class of compound is challenging owing to the lack of chromophore for conventional UV or fluorescence detection.[42]

Previous literature reviews have shown several quantitative approaches for bisphosphonates based on chromatography and non-chromatographic method. LC generally offers reliable methods characterized by sensitivity, ruggedness and accuracy. The separation efficiency of these techniques makes them a useful tool not only for assay purposes, but impurities profiling and metabolites analyses as well. Majority of LC assays such as RPLC and IPLC require pre- or post-column derivatization reactions. Derivatization is compulsory for sensitive analysis for bisphosphonates without a specific detectable property. Still, method without a derivatization is generally preferred to avoid time loss and huge risk of variation caused by derivatization of drug.Calcium precipitation is very typical for the bioanalysis of bisphosphonates;however, the preparation step is very tedious. In some cases, where manipulation of the pH of mobile phase fails to separate mixtures of very polar drug, IPLC is one of the most popular approaches to achieve efficient separations. Using the common C18 column, an amphiphilic anion or cation, such as alkyl sulphonic acid or salt and alkyl quaternary amine, is added to the mobile phases to enhance the retention of polar analytes. Ion chromatography with indirect UV detection method developed by C. Fernandes for the determination of etidronate, clodronate, pamidronate, and alendronate in bulk material and pharmaceuticals are simple, and able to demonstrate good precision, accuracy, and specificity. The methods are rapid and utilizing buffers, detector and silica column that are commonly found in laboratories. [41,43] One thing to take note that, the usage of ion-pairing agent will cause ion suppression which will render the method unsuitable for mass spectrometry.

GABA Analogues: Gabapentin, pregabalin and vigabatrin are structural analogues of cyclic gamma-amino butyric acid (GABA), the primary inhibitory neurotransmitter in the central nervous system. Gabapentin is originally developed for the treatment of epilepsy, however it has many off label uses in other conditions, such as relieving neuropathic pain and prevention of frequent migraine headaches, post-operation neuropathic pain and nystagmus. It also acts as mood stabilizer in the treatment of bipolar disorder. Pregabalin is approved for the treatment of partial seizures in patients with epilepsy and for the treatment of neuropathic pain in Europe. Though there are raising concern of abuse potential for these drugs especially in Sweden and Finland. Vigabatrin is an antiepileptic of newer generation, mainly for treatment of focal seizures and secondarily generalized seizures, as well as West Syndrome with tuberous sclerosis. Similarity in structures of GABA, gabapentin, vigabatrin and pregabalin can be seen in Figure 3.Vigabatrin, pregabalin, and gabapentin have small size, lack of chromophore and zwitterionic. For instance, gabapentin is highly water soluble and is zwitterionic at physiological pH (pKa value of 3.68 and 10.7). The existence of both amino and carboxylic groups in these drugs enable a series of derivatization reactions to take place. After derivatization, numerous analytical approaches such as GC, GC-MS, HPLC, CE, fluorometry and spectrophotometry can be done for their determination in pharmaceutical preparations and biological samples. Majority of analytical protocols were based on spectrophotometry (43%), followed by HPLC methods (33%). Up to 65.5% of the published protocols include a specific derivatization procedure for ultraviolet-visible (79.5%) or fluorescence (15.4%) detection.[52,53] Some of the studies are being summarized in Table 3.Many HPLC assay procedures for gabapentin analysis are using similar approach, involving a derivatizing agents like O-phthaldehyde (OPA), separation in acidic mobile phases and fluorometric detection. The major drawback of this application is that OPA derivative was only stable for 25 min and thus not suitable for routine clinical monitoring. Other methods that involves derivatization with chemical reagent were time consuming and the stability of the reaction products depends on experimental conditions such as pH, temperature and

reaction time. Recently there are increase in development of RP-HPLC method for quantification of gabapentin and pregabalin in pharmaceutical formulation to simplify the process and reduce the use of derivatives. For determination of GABA derivatives in biological fluids, sophisticated methods such as methods based on LC-MS-MS were employed since they are sensitive and reliable. However, the instruments are too expensive and unavailable in most of clinical laboratories. Furthermore, the carry-over and ion suppression effects are main analytical problems of LC-MS methods which are against the routine use of these methods. The GC methods involve complex sample preparation involving double derivatization process to improve the volatility and avoid column interactions. Even though CE has advantages such as simplicity and wide applicability, HPLC method is more precise, reproducible, and sensitive than the former. Fluorescence spectroscopy provides high level of sensitivity while achieving a wide concentration range, but it is still less accurate and less specific than HPLC methods. Due to the absence of fluorescent nature, these GABA analogues can be determined only after performing a suitable derivatization protocol. Some researchers omit such procedures, in order to accelerate analysis due to the lack of requirement for high sensitivity in bioanalysis. [53]More direct detection methods such as attenuated total-reflection FTIR spectrophotometry for quantitative analysis of vigabatrin in capsules was being explored, with advantage of being a simple and rapid determination method.

D-penicillamine

D-penicillamines arechelating agent which is used as disease-modifying anti-rheumatic drug. They could also be applied in cystinuria and in heavy metal poisoning treatment. This agent is an α-amino acid metabolite of penicillin, although it has no antibiotic properties. However, the L-form of penicillamine is toxic. The novel D-penicillamine, bucillamine [N-(2-mercapto-2-methylpropionyl)-L cysteine] is a cysteine-derived analog that contains two sulfhydryl groups. These sulfhydryl groups react with and neutralize toxic oxygen products that participate in the chemical reaction that leads to reperfusion injury, potentially protecting tissue from damage. [68,69]Their structures are shown in the figure 4 below.Under prolonged treatment, D-penicillamine will induce many undesirable effects including hypersensitivity, nephrotic syndrome, and myasthenia. This shows the necessity of sensitive and selective assay on human biological samples to detect possible toxicity. Due to the lack of chromophore in D-penicillamine molecule, direct UV or fluorescence spectrophotometry cannot be used for its analysis. Most of the methods are based on GC or HPLC chromatography with necessary derivatization process in order to increase specificity and sensitivity of the assay, then followed by spectrophotometric methods. Most of the reported colorimetric methods are time consuming or lacking selectivity due to the problem of interference with degradation product of coloring agents. [69] Another challenge faced in the quantitation of D-penicillamine in biological samples is the complication caused by the occurrence of many different forms; free thiol; internal disulfide; mixed disulfide with cysteine; metabolite S-methyl-D-penicillamine; and D-penicillamine bound to plasma proteins.[70]The quantification of D-penicillamine in complexed forms with metal ion while examining its chelating property can be done by recently developed HPLC-indirect UV method. The key improvement is that this method may provide faster and more accurate analysis in bulk drugs as well as in formulation form, for routine use in the future. However, this is only unique to this drug class where it is utilizing its property as a metal chelating agent. Direct spectrophotometric method is still more preferred in bulk and formulation analysis. With the commercial software involving chemometric approaches, PCR+ and/or PLS, FTIR methods existing in the literature could perform quantification within 5–10 minutes, including the sample preparation and spectral acquisition.

DISCUSSIONS

The efficacy, safety and economy of drug therapy are extremely important issues not only from the point of view of public health, but also the financial power of a country.

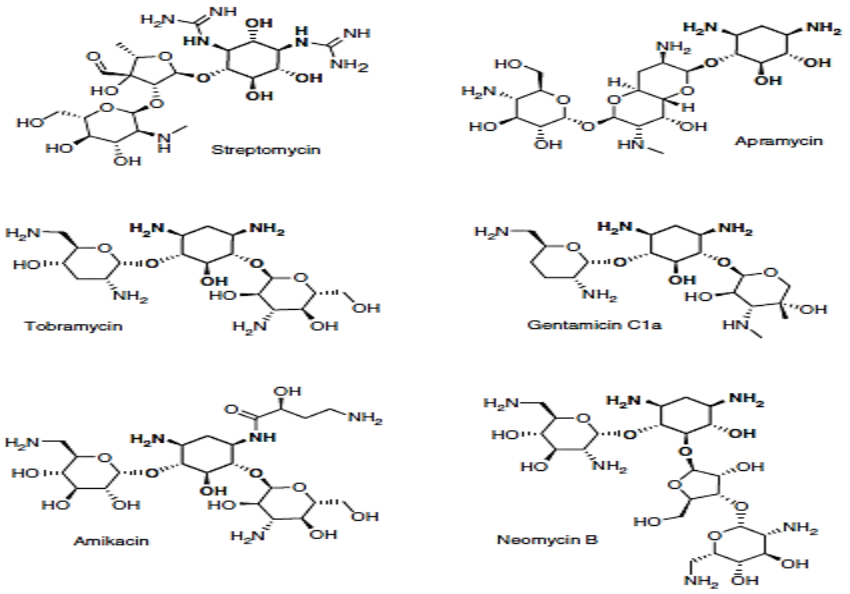


Figure 1. Structures of important AGs.

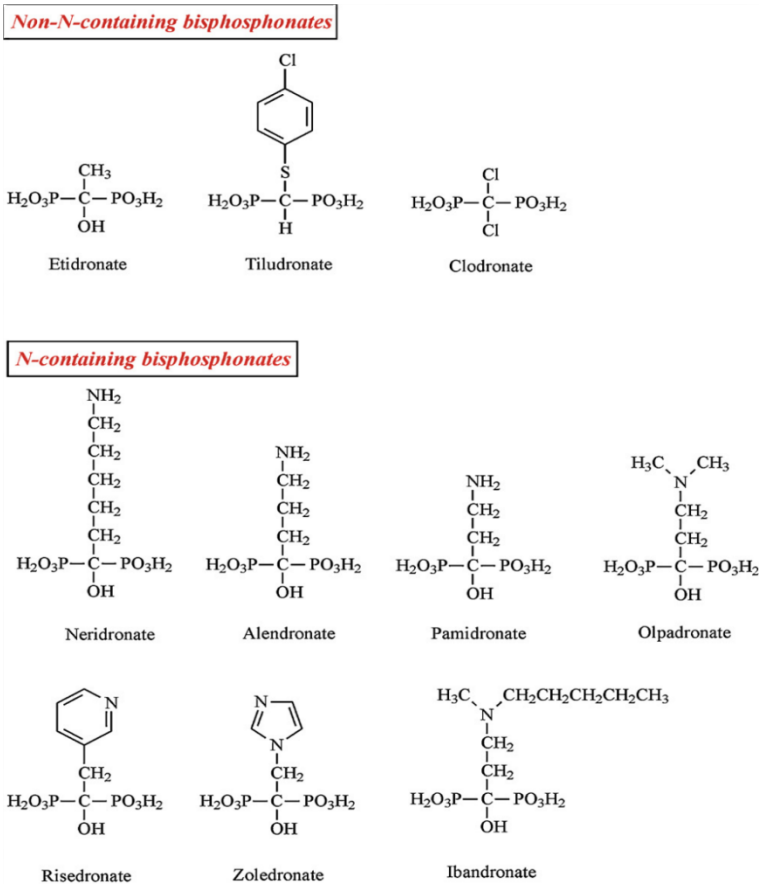


Figure 2. Structures of bisphosphonate drugs

Table1. Summary of some of the analytical methods developed for AGs.

Analytical assay	Condition	AGs involved	Specification	Limitation	Reference
Microbiological assay	USP, BP, European pharmacopoeia method for determination of AGs in bulk material and formulation, by agar diffusion or turbidimetry	Most AGs	Easy to perform, no special equipment needed, inexpensive, require small sample size (25 µl)	Relatively longer analysis time (18-24 hours), lacks specificity and sensitivity,requires carefully controlled conditions duringpreparation	[12,17,18]
Radioimmunoassay	Commercially available kits available	Most AGs	Specific quantification in serological samples for TDM with high sensitivity	Labor intensive, cross-reactivity issue, requires expensive β-counting equipment, radioactive material disposal	[12,17]
Spectrophotometry	UV detection with gentamicin-ninhydrin complex in biodegradable polymer encapsulated bmicro-particle	Gentamicin	Alternative method for quantification without undermining sensitivity	Complex fabrication process of gentamicin encapsulation	[19]
	Picric acid and 2,4-Dinitrophenol as derivatization agents	Amikacin	Tested for wide range of commercial formulation	Hazardous reagents	[20]
	Fourier Transform Infrared derivative spectroscopy	Amikacin	Reagent-free test	Not suitable for complex matrix with interfering compounds	[21]
HPLC (RPLC/IPLC)	PAD	Gentamicin Tobramycin	High sensitivity and efficiency for impurity study	Difficult in routine use due to problematic signal stability, solvent not compatible with direct	[22,23,24]

		Amikacin		coupling to MS detection, require skilled personnel	
	ELSD detection	Gentamicin	Convenient and low-priced detector, avoid the need for derivatization	Relatively low sensitivity, non-linear detector response and occurrence of non-reproducible spike peaks at high analyte concentrations	[25]
	Direct UV detection at high pH	Plazomicin Kanamycin A	Avoid preparation steps that are tedious and time-consuming, reduce reaction incompleteness variability	Short life span of stationary phase in column due to high alkaline pH	[26,27]
	Direct MS detection with porous graphite column	Gentamicin Streptomycin	Avoid complex derivatization, better than HILIC column	Specific column like porous graphite column is rare	[28,29,31]
	Direct MS detection at high pH	Tobramycin	Avoid complex derivatization	Alkaline pH deteriorates stationary phase in column faster	[16]
	Tandem MS (MS/MS)	Tobramycin	Provide highest sensitivity results, short analysis time (3.5 to 10 minutes)	Use of ion-pairing agent suppresses sensitivity	[32,33]
	CAD detection	Amikacin Apramycin	Performance superior to ELSD	Choice of mobile phase additives is limited; volatile mobile phases must be used	[29,34,35,36]

		Streptomycin Gentamicin			
CE	UV detection with Borate complexation	Streptomycin Tobramycin	Able to detect wide range of AGs	Lack of sensitivity, only applied to analysis of pharmaceutical samples	[37,38]
	Pre-column derivatization and argon-ion laser-induced fluorescence detection	Kanamycin, Bekanamycin, paromomycin, tobramycin	Good quantification method in biological samples for TDM, derivatization improve sensitivity	Long preparation steps	[39]

USP- United Stats Pharmacopeia. BP- British Pharmacopeia

Table 2. Part of the analytical methods developed for bisphosphonate drug detection

Analytical instrument	**Condition**	**Bisphosphonate involved**	**Specification**	**Limitation**	**References**
Spectrophotometry	7-chloro-4-nitrobenzofurazon (NBD-Cl) and 2,4-dinitrofluorobenzene (DNFB) derivatization	Alendronate	Inexpensive analytical procedures with common reagents	Complex and long reaction rate may cause incompleteness in reaction, require high temperature	[44]
	FTIR, Raman, NMR	Risedronate	Study on adsorption on nanocrystalline apatite	Does not study on quantification	[45]
HPLC	Co-precipitation with calcium phosphates, automated pre-column derivatization with	Alendronate	Improved detection of drug in human urine and plasma samples	Complex procedures increase variability in reaction process, uses of non-environmentally	[46]

	cyanide or naphthalene-2,3-dicarboxyaldehyde (NDA) reagents, fluorometric and electrochemical detection			friendly regents like cyanide.	
	MS detection, double derivatization firstly with isobutyl chloroformate (IBCF), then with trimethyl orthoacetate	Alendronate	Higher sensitivity and selectivity method compared to GC-MS	Complex procedure with multiple derivatization may cause incompleteness in reaction	[47]
	MS/MS, diazomethane derivatization	Alendronate	Avoiding the tedious calcium precipitation step in HPLC- fluorescence detection	High non-volatile salt content of drug requires derivatization process to enable tandem MS detection	[48]
	Ion-pairing with indirect reflective index (RI)/ UV detection	Alendronate, etidronate, and clodronate	Simple and suitable for routine analysis	The detection limit of IC-RI detector is better than IC indirect UV	[42,43,49]
	ELSD, ion-pairing with ammonium acetate buffer	Ibandronate	Solves retention issue due to more than one ionizable group	Solvent unsuitable for direct MS detection, may cause ion suppression	[50]
CE-UV	Complex formation withCuSO, (1:1 stoichio-metry), direct UV detection on alendronate –Cuchromophore.	Alendronate	Minimum sample preparation, requires small amount of organic solvent-free electrolyte, efficient separation	Poorer precision as compared to HPLC method	[51]

Table 3. Part of analytical methods developed for GABA analogues determination.

Analytical method	Conditions	GABA analogue involved	Specification	Limitations	References
Spectrophotometry	Reaction with ninhydrin and pi-acceptors	Gabapentin	Use of simple and inexpensive chemicals	Reaction variability concern	[54]
	Fluorescent derivatives from reaction with fluorescamine in borate buffer of pH 8.2	Gabapentin, vigabatrin	Short derivatization time (1 minute) at room temperature, easy sample handling, and the stability of the reaction product	Lower accuracy and less specificity than sophisticated method	[55]
	Chloroform soluble ion-association complexes with bromocresol green/ bromothymol blue in a phosphate buffer of pH 4.0	Gabapentin	Simple procedure for formulation analysis, easily available reagents and solvents	Non-environmentally friendly solvent used	[56]
	Reaction with NBD-Cl	Pregabalin	Simple, applicable for analysis of bulk material and capsule form.	Lower accuracy and less specificity than sophisticated method	[57]
	Complexation with p-dimethylaminobenzaldehyde (PDAB) in an acidic medium	Pregabalin	Determination in bulk and capsule dosage form	Lower accuracy and less specificity than sophisticated method	[58]
HPLC	Fluorescence detection, OPA and 3-mercaptopropionic acid / NDA as pre-column derivatization agent	Gabapentin Pregabalin Vigabatrin	Good sensitivity and selectivity, common derivatizing agent used	Prolonged determination due to complex preparation	[59,60]
	UV detection with double step derivatisation/ pre or post-column derivatization agent/ ion-pairing	Gabapentin Pregabalin	Favourable due to instrumentation availability and reliable	Complex preparation steps	[61,62,63]

	agent		results		
	UV detection / PAD detection without derivatization	Pregabalin	An approach to simplify method	Lack of sensitivity based on presented spectra and selectivity, since pregabalin was eluted too close to dead volume	[64,65]
LC-MS/MS	CAD detection using Kinetex Biphenyl column	Gabapentin	Specific, accurate and reproducible in determining potential impurities	Choice of solvent is limited and volatile mobile phases must be used, uncommon column	[66]
GC-MS	Single-step derivatization with MTBSTFA	Gabapentin	Good sensitivity, with sample derivatization	MTBSTFA remaining after derivatization affected mass selective detector cleanliness	[53]
	Methylation derivatization with N-trimethylsulfonium hydroxide (TMSH)	Gabapentin, vigabatrin	Good sensitivity with sample derivatization	Require complex preparation to improve the volatility and avoid column interactions	[67]

Table 4. Part of analytical methods developed for D-penicillamine determination.

Analytical method	Condition	Drugs involved	Specification	Limitation	References
Spectrophotometry	Colour reaction with sodium1,2-naphthoquinone-4-sulfonic (NQS)	D-penicillamine	Does not need any expensive apparatus, simple, rapid for analysis in common laboratory	Unstable complex formed	[71]
	Direct FTIR quantification	Bucillamine	Fast and accurate	Could not provide	[72]

	with chemometric software (PCR+ and/or PLS)		determination in pharmaceutical formulations, without sample pre-treatment	good sensitivity for clinical use/TDM	
HPLC	Cation-exchange column, with ninhydrin derivatization	D-penicillamine	Useful in determining all forms and metabolites of D-penicillamine	Extensive manipulation on sample undesirable for clinical use	[73]
	Fluorescence detection, with derivatization agents such as N-(1-pyrenyl) maleimide (NPM)	D-penicillamine Bucillamine	High sensitivity in quantification of liver, kidney, brain and plasma samples	Certain agents have specific reaction conditions, shorter time of stability after derivatization	[70,74,75]
	UV detection, complexation with Fe^{2+} and Cu^{2+} metal ions.	D-penicillamine	Fast and cost-effective in bulk drugs and formulation analysis	pH of the solution had significant influence on complexation process	[76]
	Tandem MS detection with isobutyl acrylate (IA) as derivatizing agent	Bucillamine	Improved reaction time and less volatile as compared to previous studies	Agent hazardous to environment	[77]
GC	MS detection or electron capture detection	D-penicillamine Bucillamine	High sensitivity and specificity	Time consuming, too specialized for clinical setting, stability issue	[69]

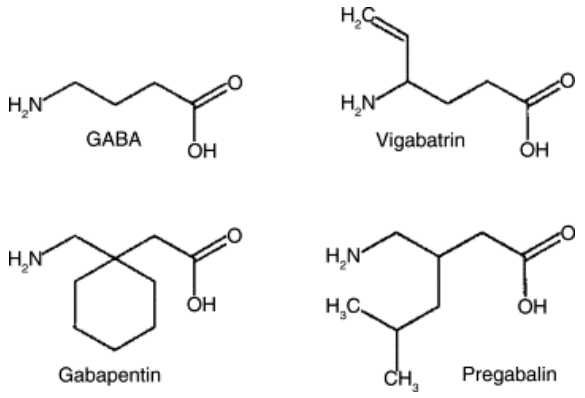
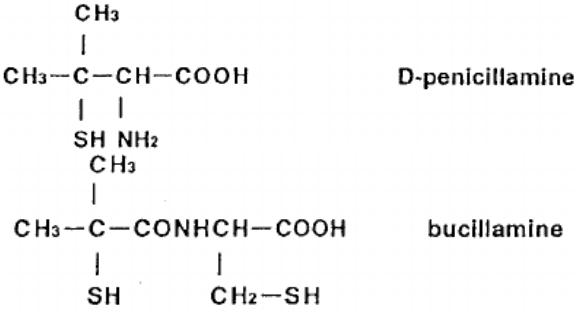


Figure 3. Chemical structure of GABA, vigabatrin, gabapentin and pregabalin

From the prospect of Sustainable Development Goals (STGs) and Green Chemistry, future recommendations are summarizedbelow with six main points. [78,79]

Cost

The instrument and detector choices are largely affected by the availability and financial aspects, especially in developing countries. For example, HPLC with CAD/PAD has gained popularity in the determination of non-chromophore drugs without derivatization process and excessive steps. However, not only these sophisticated detectors require a high cost, training of personnel will require extra expenses too. The novel AuNP colorimetric method in kanamycin A and streptomycin



detection requires expensive instrumentation as well. This will increase the burden of cost for small scale sectors like academic researchers or industry field. Goal 3 of STGs promotes the use of analytical methods which are rapid, efficient and cost-effective to increase health financing and the development of public and private health sector in developing countries, especially in least developed countries and small island developing States. Methods with complex derivatization process which prolonged runtime are to be avoided in clinical use, which is concurrent with the eighth principle of Green Chemistry. Most usually prefer methods that utilize buffers, detector or column that are commonly found in laboratories.

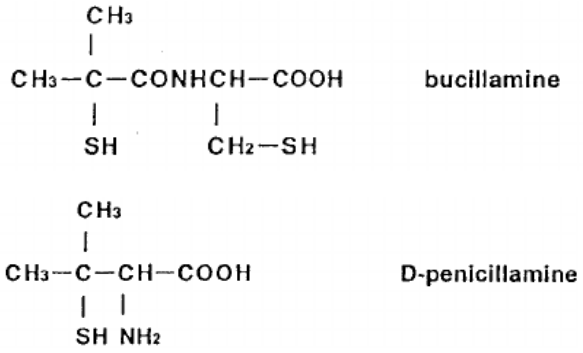


Figure 4. Chemical structure of D-penicillamine and its analogue bucillamine

Environment

Even though HILIC column could provide better retention of AGs due to extreme hydrophilic property, HILIC is potentially an environmentally less friendly technique as it consumes much larger volumes of organic solvents. This has gone against our mission as stated in Goal 6 of SDG. Goal 6 of SDG aims to improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials. Green chemistry also proposed development of less hazardous methodologies that use or generate substances which may pose little or no toxicity to the environment.Use of hazardous derivatizing agents such ascyanide and 2,4-Dinitrophenol should be reduced. Thus, more direct method such as FTIR and Raman spectroscopy are utilised more effectively for quantitative analysis of pharmaceuticals, which do not require derivatization or no addition of solvent.

Time

Most methods involve derivatization process would require a longer time to obtain result, including different reaction time depending on reagents used. Complex procedure with multiple derivatization might cause incompleteness in the reaction,long waiting times for reaction. Quick and simple methods are often a challenge to develop for patient

biological samples testing so that it can be carried out in more efficient manner, due to interference of multiple organic and inorganic substances in blood, urine or tissue samples.

Routine analysis

There is a need for research to establish affordable sophisticated analytical methods which can rapidly evaluate qualities of large quantity of drug for quality control in pharmaceutical industry. UV/Vis spectroscopy is often used for routine analysis to perform quick analysis for bulk materials and formulations. However, for drugs that lack of a chromophore, the widespread UV/Vis detection system will not be suitable unless analyte derivatization is performed. The main drawbacks of conducting derivatization are long time consumption, labor intensive, and large overall variability due to extra sample preparation steps. Thus, more direct spectrophotometric method which do not require derivatization or addition of solvent should be studied further to reduce cost-burden and support growth of micro-, small- and medium-sized enterprises for the need of quality control and regulatory requirements, as proposed in eighth goal of SDG. This is to support developing countries to strengthen their scientific and technological capacity to move towards more sustainable patterns of consumption and production. Analytical procedures also need to be designed further to introduce real time in-process monitoring and control for processes that utilize or generate hazardous outcome, as proposed by the eleventh principle of green chemistry.

Field applications

Spectrofluorimetry and spectrophotometry are techniques of choice in research laboratories, hospitals and pharmaceutical industries due to its low cost and inherent simplicity. Development of quality, reliability and sustainable analytical method/instrument are important to support economic development in public/private health sector, also in particular to mention Pharmaceutical industries to provide affordable quality medicines and access to high quality assurance for all drugs, as quoted from the ninth and twelfth goal of SDG. Pharmaceutical companies, especially large and transnational companies are encouraged to adopt sustainable practices and to integrate sustainability information into their reporting cycle. There are few exemplary achievements by major pharmaceutical companies like Merck, Pfizer etc in having industrial change to green chemistry, mentioned by Anastas P. and Eghbali N. in their critical review. In doing so, Green Chemistry has shown that through innovation companies can be economically more profitable and more environmental benign at the same time. [80]

Regulatory requirements

New generation pharmaceuticals are administered in lower doses and require highly sensitive methods. Mass spectrometry detection hyphenated to LC has become a powerful analytical tool forroutine analysis of most molecules including weak UV absorbing analytes of recent. However, most of the mobile phases are not compatible to MS detectors and many small molecules are not ionizable by electrospray ionization. Although sample derivatization can be used to introduce a chromophore group to enable UV or MS detection, as it significantly increases sample preparation time and will increase variability associated with incomplete conversion. As MS detection becomes more widely available and its quantitative performance improves, this will probably replace spectrophotometric and electrochemical detection in many applications. Authorities haveplayed major role in ensuring that the field of chemistry is practiced in a way that impact on people and the planet in the positive way. As a result, SDG goals and Green Chemistry principles were created to provide a referral guidelines to pursue more environment friendly and cost-effective analytical methodologies for not only pharmaceutical field, but all life cycle processes.

CONCLUSIONS

This extensive review revealed that it is necessary to choose appropriate analytical method feasible, cost effective, less time consuming, non-destructive and sample recovery for various classes of no chromophore

drugs. Analysis cost is the major contributor to any pharmaceutical industry since every industry needs to be well equipped with sophisticated instruments, which incur high cost due to sophistication, laboratory set up and cost. Small -scale industries look for validated, simple and economical analytical method for routine analysis of batch to batch drug products for regulatory approvals and release into market timely as per demand and supply without compromising the quality of medicines. Drugs with complicated structural features, no chromophore functional groups pose difficult tasks for an analyte to develop simple and direct analytical method except dependent on sophisticated analytical techniques like HPLC and HPLC integrated with mass spectrometer (LC-MS). Though sophistication embedded with latest software in HPLC is not sufficient to analyze these no chromophore drugs due to lack of UV-detector response in HPLC. MS detectors with HILIC system detects these non-chromophore drugs, by compromising the complex analytical process, poor reproducibility of results during analysis, column deterioration due to high alkaline pH conditions, long retentions of analyte in column.

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