Review Article

CHEMICAL DERIVATIZATION METHODOLOGIES FOR UV-VISIBLE SPECTROPHOTOMETRIC DETERMINATION OF PHARMACEUTICALS

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ABSTRACT

Pharmaceuticals can be classified into inorganic, organic compounds as well as excipients. The need to have a readily adaptable method for the quality control of these compounds has led to the development of a wide range of reactions and procedures. Majority of these pharmaceuticals lack adequate chromophores which can permit analysis at wavelength regions beyond the non-specific UV-region of the electromagnetic spectrum. Thus derivatization reactions are carried out to convert these pharmaceuticals to readily determinable compounds whose properties and concentrations can be related to the original compound. An avalanche of reaction has been reported in the literature for the assay of pharmaceuticals. This paper presents an overview of these reactions. The need to have simple methods for the analysis of pharmaceuticals, in spite of the improvement in modern-day technology, will continue to make these derivatization methodologies relevant in the quality control of these compounds especially in poor-resource economies.

Keywords: Spectrophotometry, Derivatization, Chemical reactions, Organic compounds.

1.0 INTRODUCTION

In many instrumental techniques, it might be desired to assay particular compounds in forms that are readily handled to improve sensitivity or selectivity. Usually conversion of functional groups within the molecule to others more readily adaptable to the technique at hand is preferred. This procedure called derivatization or derivative formation is applied in UV-VIS spectroscopy, gas chromatography (GC) and high performance liquid chromatography (HPLC).

In UV-VIS absorption spectroscopy, although many organic compounds absorb quite strongly, only a limited number of inorganic ions do, and it is the normal procedure of inorganic absorption spectrophotometry to add a reagent species to the solution of the inorganic ion that reacts with it and, in the process, bring about a marked change in the spectral absorption characteristics of the reagent[1]. In such reactions, colours are produced and having selected the λ_{max} by a spectrophotometer, colorimeters are used for quantifying the amount of absorbing species. While this method has been popular for inorganic ions such as iron (II) [1, 10-phenanthroline complex], Ni (dimethylglyoxime), Si (ammonium molybdate), Zn (dithizone), Co (thiocyanate)[2], colorimetric measurements of drugs and other organic compounds are now common after specific derivatization procedures. Thus, the charge-transfer complexes commonly used for inorganic ions are now used in colorimetric assessment of ampicillin[3] and antimalarials[4]. Although very few reactions are specific for a particular substance, many reactions are quite selective, or can be rendered selective through the introduction of masking agents, control of pH, solvent extraction, adjustment of the oxidation state, or prior removal of interferents. Both the colour-developing reagent and the absorbing product must be stable for a reasonable period of time[1].

Chemical derivatization fall under the category of indirect spectrophotometric analysis and the compounds of interest are often converted to those with different spectral properties. One peculiar procedural step is the utilization of excess reagent in order to ensure complete conversion of the analyte and thereby increasing the dynamic working range possible. Majority of chemical derivatization reactions involve conversion of the analytes to molecules with longer chromophores hence absorption in the visible region and or conversion to species with hyperchromic absorption. Chemical derivatization may be adopted in instances where the analyte absorbs weakly in the UV region as is common with most drugs, where interference by irrelevant absorption is present, where there is a need to improve selectivity of the procedure or when cost implications will favour adoption of a colorimetric method to that of a UV-VIS spectrophotometer[5].

2.0 GENERAL CLASSIFICATIONS OF THE REACTIONS

A wide range of reactions have been adopted for the chemical derivatization of inorganic and organic pharmaceuticals. Majority of these reactions are colour-producing reactions. The reason for the formation of such derivatives is not farfetched as many of these pharmaceuticals lack extensive chromophores. Most direct spectrophotometric determinations of organic pharmaceuticals, in particular, are carried out at wavelength regions in the range 200-330 nm. No matter how poorly endowed interference from dosage form excipients, solvents and extraneous matter is common in this region. Thus the challenge is to carry out derivative

formation which shifts the absorption maximum to mid-spectrum region and the colorimetric range (380 – 800 nm).

Absorption spectroscopy provides a useful tool for qualitative analysis. Identification of a pure compound by this method involves an empirical comparison of the details of the spectrum (maxima, minima and inflection points) of the unknown with that of the pure compound. A close match is considered good evidence of chemical identity, especially if the spectrum of the unknown contains a number of sharp and well-defined peaks. Absorption spectroscopy is one of most useful tools available to the chemist for quantitative analysis. This is because it has wide applicability, high sensitivity, and moderate to high selectivity, good accuracy and ease as well as convenience.

Colorimetric methods can selectively transform a drug, its impurity, or a metabolite so that the spectrum is shifted to the visible region and away from interference caused by another drug, formulation components, or biological substances, thereby conferring a further degree of specificity. Moreover, a drug with little or no useful absorption can be more sensitively determined by modifying it to a more highly absorptive chromophore[6].

Prominent pharmacopoeial examples of colorimetric measurements include tetrazolium assay of corticosteroids, the assay of folic acid involving hydrolysis, diazotization and coupling with N-(1-naphthyl) ethylenediamine, as well as the reaction of penicillins with imidazole and mercuric salts.

In the light of the foregoing it is imperative some conditions are fulfilled by the derivatizing reagents, the reaction procedure as well as the product resulting from the derivatization process. Parameters which require critical consideration for successful colorimetric analysis are; selectivity of colour reagent for drug molecule itself, solvent, pH, temperature, reagent excess, order of mixing reagents, ageing of reagents as well as careful assessment of the absorbance properties and stability of the chromophore generated.

Some reactions have enjoyed wide applicability in chemical derivatization for the UV-VIS spectrophotometric determination of pharmaceuticals. The major reactions are azo dye derivatization, hydrazine derivatization/Schiff base formation, organic charge transfer reactions/complexation, ion-pair formation, complexation reactions, oxidation-reduction reactions and some miscellaneous methods.

2.1 Azo dye derivatization

Azo dye derivatization perhaps constitutes the most widely applied reaction for the chemical derivatization of drugs. Azo dyes are characterized by the presence of the diazo linkage which brings two rings into conjugation and thereby extending the maximum wavelength (λ_{max}) to the visible region (Figure 1). As a result of such dye formation, lower detection limits are obtained while at the same time some measure of selectivity is afforded. Azo dyes are produced by the diazo coupling reaction of a diazonium salt with a neutral, activated or deactivated skeleton. The rate of such coupling reaction and hence the applicability of the derivatization procedure depends on some structural factors related to the diazonium ion. The diazonium ion acts as an electrophile and the formation of an azo dye and the avidity as well as rate of formation are determined by the reactivity of the diazonium ion. With the presence of strong electron-withdrawing substituents on the diazonium ion, the diazo coupling reaction becomes fast and reaction with a neutral or deactivated skeleton is possible.

The formation of an azo dye proceed by two main steps; preparation of diazonium salt and coupling of the latter with a suitable compound.

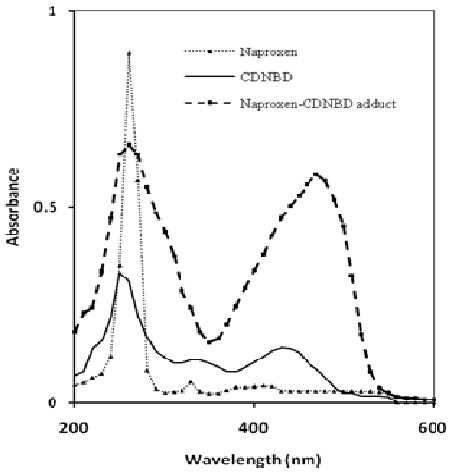
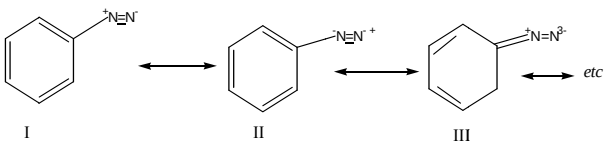


Figure 1: Colorimetric determination of naproxen following azo dye derivatization with CDNBD[35]

2.1.1 Diazonium salts

Each class of amines yields a different kind of product in its reaction with nitrous acid, HONO. This unstable reagent is generated in the presence of the amine by the action of mineral acids on sodium nitrite. Peter Griess first discovered the original reaction in 1858[7]. When primary aromatic amines are treated with nitrous acid, diazonium salts are formed. The reaction also occurs with aliphatic primary amines, but aliphatic diazonium ions are extremely unstable, even in solution. Each class of amines yields a different kind of product in its reaction with nitrous acid, HONO. This unstable reagent is generated in the presence of the amine by the action of mineral acids on sodium nitrite. Aromatic diazonium ions are more stable, because of the resonance interaction between the nitrogens and the ring.

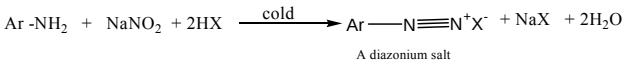


Incidentally, structure I contributes more to the hybrid II, as shown by bond-distance measurements. In benzenediazonium chloride, the C-N distance is ~ 1.42

Angstroms and the N-N distance ~ 1.08, which values fit more closely to a single and a triple bond than to two double bonds[8].

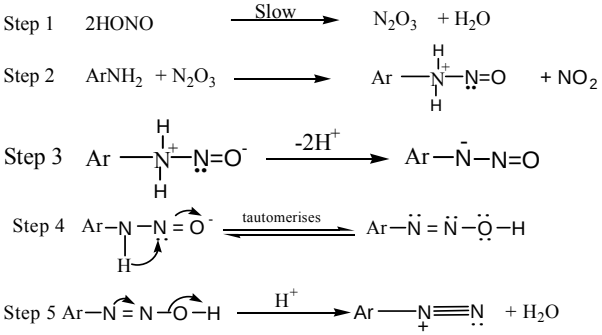
2.1.1.1 Preparation of diazonium salts

The preparation of diazonium salts is by a process known as diazotization and a salt of the ion results depending on the mineral acid used.

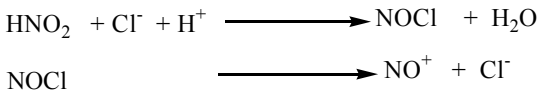


X^- could be HSO_4^- or Cl^- if H_2SO_4 or HCl is used.

Diazonium salts are usually prepared in aqueous solution and used without isolation. The aromatic diazonium salts are stable only at low temperatures, usually only below 5°C, though more stable ones, such as diazotized sulphanilic acid, are stable up to 10 or 15°C. The stability of aryl diazonium salts can be increased by "crown ether" complexation[8]. For aromatic amines, the reaction is very general. Halogen, nitro, alkyl, aldehyde, sulphonic acid groups do not interfere. Despite the fact that diazotization takes place in acid solution, the actual species attacked is not the salt of the amine, but the small amount of the free amine present. It is because aliphatic amines are stronger bases than the aromatic amines that the former are not diazotizable below pH 3 since very minute free amine will be available at this pH. In dilute acid the actual attacking species is N_2O_3, which acts as a carrier of NO^+. Evidence is that the reaction is second order in nitrous acid and, at sufficiently low acidities; the amine does not appear in the rate expression. Under these conditions the mechanism is[8]:



Other attacking species can be NOCl, $H_2NO_2^+$, and at high acidities even NO^+. Nucleophile (e.g. Cl^-, SCN^-, thiourea) catalyse the reaction by converting the HONO to a better electrophile, e.g.



On the industrial scale, preparation of diazonium involves adding one equivalent of aqueous sodium nitrite to the resulting mixture at 0-5°C, the exothermic nature of the reaction combined with the heat sensitivity of most diazonium salts, makes it necessary to provide cooling, usually by direct addition of ice[9].

Other commonly adopted methods of diazotization are described below [9, 10]:

(i) Inverted or indirect method*:* In this method mixed alkaline solutions of a metallic nitrite and a salt of a sulphonated or carboxylated arylamine are run into excess of cold mineral acid. Commonly used for amino acids such as sulphanilic, naphthionic and aminobenzoic as well as some weakly basic amines.

(ii) The arylamine is dissolved in a concentrated acid (sulphuric acid, phosphoric acid, glacial acetic acid) and diazotized with nitrosylsulphuric acid. It is commonly used for weakly basic amines or the so-called intractable amines; whereby the basicity of the amine is so much reduced that it is no longer soluble in aqueous acids because it's salt suffers complete hydrolytic dissociation. The nitrous acid is introduced either by powdering in the solid nitrite with stirring or as a nearly saturated aqueous solution, or may be first dissolved in the concentrated H_2SO_4. The nitrosylsulphuric acid however does not liberate nitrous acid in sufficient quantity and a weaker acid such as orthophosphoric acid is added to dilute the medium.

(iii) The arylamine is dissolved in nitric acid and metabisulphite is added, thus producing nitrous acid which diazotizes the amine. This is the Witt's method and used for weakly basic amines not easily nitrated or oxidized.

(iv)The arylamine salt is suspended or dissolved in water or alcohol and treated with gaseous nitrogen trioxide; this is the Griess's method, which is rarely used now.

(v) The arylamine salt is dissolved in water, or suspended in alcohol or inert solvent and treated with an alkyl nitrite or an ester of nitrous acid, e.g., nitrosyl chloride. Commonly, referred to as the Knoevenagel method. Used for effecting diazotization so that the solid diazonium may be isolated.

2.1.1.2 Reactions of diazonium salts

The large number of reactions undergone by diazonium salts may be divided into two classes:

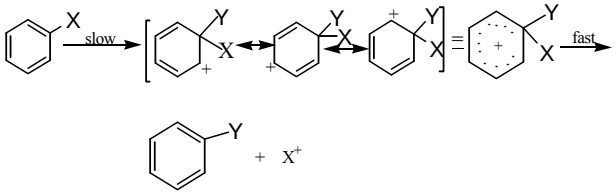
(i) Replacement reaction; in which nitrogen is lost as N_2 and some other atom or group becomes attached to the ring in its place.

(ii) Diazo-coupling reaction; in which the nitrogen is retained in the product [11].

Replacement of the diazonium group is the best general way of introducing F, Cl, Br, I, CN, OH and H into the aromatic ring. This reaction is generally referred to as de-diazoniation e.g. chloro-de-diazoniation, nitro-dediazoniation in which negatively charged Cl- and NO_2 - (from $NaNO_2$) replaces N_2 on the ring respectively[11].

Under the proper conditions, diazonium ions react with certain aromatic compounds to yield products of the general formula Ar-N=N-Ar, called azo compounds. In this reaction, known as *coupling reaction*, the nitrogen of the

diazonium group is retained in the product, in contrast to the replacement reactions, in which nitrogen is lost. The reaction on the aromatic substrate (coupler) proceeds by aromatic electrophilic substitution of the diazonium ion on sites on the ring predetermined by substituents present on the coupler. Usually the reaction proceeds *via* the formation of a carbocation intermediate called *Wheland intermediates*, *δ-complexes, or arenium ions* (arenium ion mechanism). The first step, which is slow and rate determining, is the attack of the ring by the electrophile, followed by the fast step in which the leaving group departs as a full positively-charged species (often a proton; except in cases where a stronger electrophile displaces a weaker one already present on the ring) as shown in Scheme 1[12].



Scheme 1: Electrophilic substitution on benzene ring

The aromatic ring undergoing attack by the diazonium ion must, in general contain a powerfully electron-releasing group, generally –OH, -NR_2, or -NH_2. Presumably because of the size of the attacking species, substitution is mostly *para* to the activating group, unless that position is already occupied, in which case *ortho* substitution takes place. Typically, coupling with phenols is carried out in mildly alkaline solution, and with amines in mildly acidic solution.

Primary and secondary amines face competition from attack at the nitrogen. However, the resulting N-azo compounds (aryl triazenes) can be isomerized to C-azo compounds. An initial N-azo compound may be formed in most cases to isomerise to C-azo compound[13]. O-azo. S-azo is also possible. An example of the O-azo compound formation is the spectrophotometric determination of Metoclopromide, dapsone, *p*-aminobenzoic acid and cisapride in pure and dosage forms using acetyl acetone (penta-2, 4-dione), in alkaline medium, as coupling component[14].

Acylated amines and phenolic ethers and esters are ordinarily not active enough for this reaction, though it is sometimes possible to couple them (as well as such polyalkyalted benzenes as mesitylenes and pentamethylbenzene) to diazonium ions containing electron-withdrawing groups in the *para* position, since such groups increase the concentration of the positive charge and thus the electrophilicity of the ArN_2^+. Some coupling reactions that are otherwise very slow (in cases where the coupling site is crowded) are catalyzed by pyridine [13]. While the above considerations have been on monoazo compounds, bis-, tri-, and polyazo products are possible and are commonly used as industrial pigments.

2.1.2 Application of Azo-dye formation in colorimetric analysis

One of the properties of an azo dye is the intense colour it is associated with. Usually with the availability of a suitable substrate and formation of an azo compound, the azo bridge (or diazenediyl linkage) brings two rings into conjugation. The consequence of this is that a new compound results with light absorption in the visible range of the spectrum. Since diazonium salts will only couple with highly activated substrates, the reaction can be used for the determination of aromatic compounds containing such activated substituents as – NH_2,-NHR, -NR_2, OH, OR etc either on benzene ring or fused aromatics like naphthalenes. Pyrroles, furan and thiophenes are also known to demonstrate aromatic behaviour with the consequence that azo coupling could take place[15]. Once the colour is produced, the application involves estimation of the concentration of the coupling component by colorimetry.

The formation of the azo dye often improves the sensitivity of the determination of the compound (coupler) or drug. This is as a result of the improved absorptivity that the coloured species is associated with, in addition to lower limits of detection afforded. The reaction can also be made to be selective by appropriate selection of solvent, pH of the reaction medium or prior extraction of the drug or compound.

Colorimetric analyses of several pharmaceuticals have been described based on the formation of azo compounds. Usually, a C-azo compound is common, even though N-azo, O-azo or S-azo is possible. The reaction is particularly useful for drugs that do not have strong absorption in the visible region or for which determination in the UV region may be associated with undue interferences.

Two procedures are commonly adopted in the application of diazo coupling reaction for colorimetric analyses of pharmaceuticals:

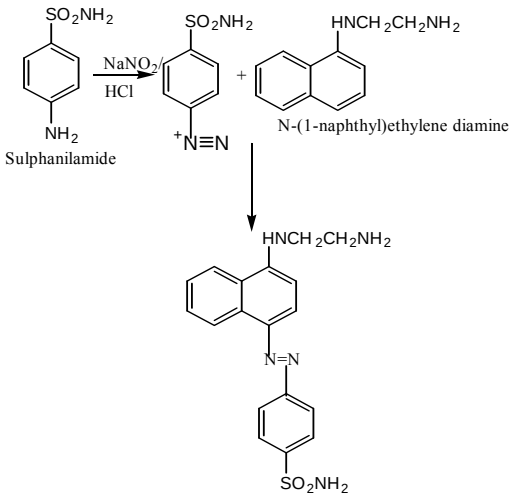
(a) Analysis of drugs involving preliminary diazotization of the drug before coupling to a suitable reagent

(b) Analyses in which the diazonium salt is the reagent.

Diazotization of drugs before coupling

In this method, the drug contains either a free primary amino group or other derivatives. Such derivatives include amide and nitro. In the former, an initial step of amide hydrolysis is done and the hydrolytic product is now diazotized using any appropriate method. The presence of a free nitro group is also exploited for their analyses. The nitro group is reduced in a one-step reaction involving zinc dust in dilute HCl or glacial acetic acid to produce an amino group. The new derivative is now diazotized. This method has been used extensively for the determination of sulphonamides. The most popular procedure is the development of a new coupling component for sulphanilamide determination by Bratton

and Marshall [16]. The method involves colorimetric determination of sulphanilamide in blood or urine by first diazotizing the sulpha drug after extraction and coupling with N-(1-naphthyl) ethylenediamine dihydrochloride, a compound which later gained popularity as Bratton-Marshal reagent (Scheme 2).



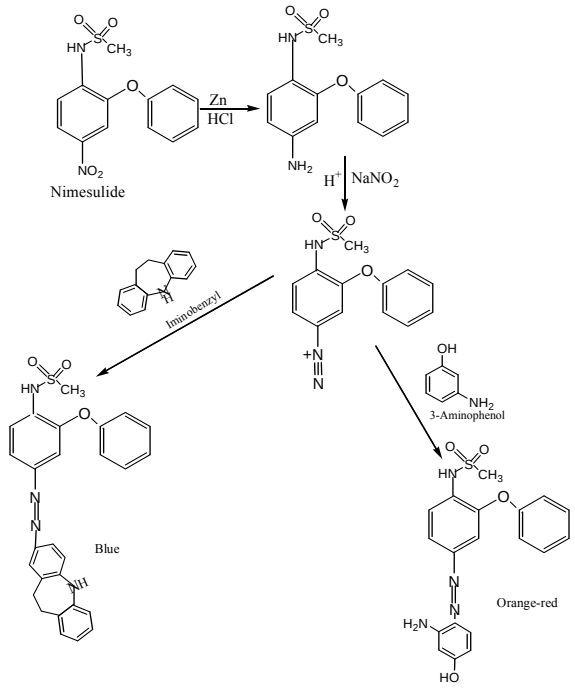
Scheme 2: Colorimetric determination of sulphanilamide using Bratton-Marshall reagent

The azo dye produced absorbed maximally at 545 nm. This coupling component was reported to have a better performance than the one earlier used by the same authors (dimethyl-α-naphthylamine i.e. N,N-dimethyl-1-naphthylamine).

Recently, the utilization of prior diazotization of the drug of interest before diazo coupling with a coupler has been adopted for the colorimetric determination of nitroimidazoles using *p*-dimethylaminobenzaldehyde as the coupling component[17]. Likewise, chromotropic acid has been used as coupling component for the determination of mosapride in pure and pharmaceutical preparations[18].

The British Pharmacopoeia (BP) identification of such drugs as metronidazole and nifedipine is based on the reduction of the nitro group. In the method for metronidazole tablets[19], a given quantity of the drug is reduced with zinc powder in HCl, cooled in ice and $NaNO_2$ solution is added. Excess nitrite is removed using sulphamic acid. 2-naphthol solution is then added to the diazotized drug. An orange-red colour is produced upon adding 2 mL of 5M NaOH. Similarly, for the identification of nifedipine, the drug is reduced with granulated zinc and then diazotized before an intense red colour is generated with N-(1-naphthyl) ethylenediamine dihydrochloride. Metoclopromide and dapsone were determined in a procedure by diazotizing their primary aromatic amines with $NaNO_2$ and then coupling with dibenzoyl methane in alkaline medium. Orange coloured azo dyes were obtained with λ_{max} 440 nm and 470 nm respectively for the drugs[20].

Nimesulide, chemically N-(4-nitro-2-phenoxyl) methane sulphonamide is a relatively new non-steroidal anti-inflammatory drug. In a recently reported method[21], the drug is reduced using zinc dust and concentrated HCl. The reduced drug is now diazotized with $NaNO_2/H_2SO_4$ in ice bath. The coupling components are iminodibenzyl (λ_{max} = 600 nm) and 3-aminophenol (λ_{max} = 470 nm). See Scheme 3.



Scheme 3 : Colorimetric analysis of nimesulide

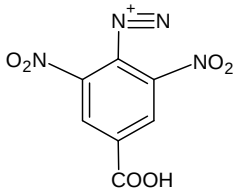
Another recent method [22] for the determination of some sulpha drugs depends on the formation of orange yellow coloured azo product by the diazotization of sulphonamides (*viz*: dapsone, sulphathiazole, sulphadiazine, sulphadimidine, sulphacetamide, sulphamethoxazole, sulphamerazine and sulphaguanidine) followed by a coupling reaction with 3-aminophenol in aqueous medium. Absorbance of the resulting orange yellow product is measured at 460 nm and is reported to be stable for 6 days at 27°C. Likewise; iminodibenzyl has been used as a novel coupling agent for the spectrophotometric determination of sulphonamide derivatives following diazotization of the sulpha drugs [23].

Diazotized compound as the reagent

This procedure has a broader application. Here, the reagent possessing a free primary aromatic amino group is diazotized using appropriate method. The reagent is now coupled with drugs possessing appropriate activated aromatic skeleton. Earliest reagents in this category include diazotized *p*-nitroaniline[24] and diazotized sulphanilic acid[25].

Diazotized *p*-nitroaniline is used in the identification of methoxamine injection by the BP 98 where a deep red colour is produced, which is extractable, by butan-1-ol. Both diazotized *p*- nitroaniline and diazotized sulphanilic acid have been used in an alkaline medium to determine ritodrine HCl and its pharmaceutical preparation [26]. In a recent application of diazotized *p*-nitroaniline, minocycline was determined in a basic medium at 420 nm[27].

In recent years, we have developed a highly reactive diazonium ion for the aromatic ring derivatization of varied skeletons. The diazonium, 4-Carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD), is prepared in nitrosyl sulphuric acid medium from the primary aromatic amine, 4- amino-3,5-dinitrobenzoic acid. CDNBD possess two nitro groups *ortho* to the diazo group and a carboxylic acid moiety *para* to the azo group. These highly electron withdrawing groups makes CDNBD highly reactive. It has been successfully applied to the determination of secondary amino derivatives; mefenamic acid[28] and diclofenac[29], reactive methylene centres (generated in-situ from artemisinin derivatives) [30] and phenol ether homologues (nabumetone[31], indomethacin[32], propranolol[33], several skeletons[34], naproxen[35], nadolol[36]). The reaction with the last group points to the high reactivity of the compound as most diazonium ions do not react with phenol ethers. This is due to the fact phenol ethers belong to the class of moderately to weakly activated nuclei and it will take exceptionally reactive diazonium ions to form azo dyes with the ethers.



CDNBD

3.0 Schiff base formation

Many colorimetric procedures are based on condensation reactions under suitable conditions between amines and carbonyls to generate Schiff's bases, hydrazones, semicarbazones or oximes.

When R" = Alkyl or aryl, the product is a Schiff's base
R" = NH_2 (hydrazine), the product is a hydrazone
R" = $NHCONH_2$ (semicarbazone), the product is a semicarbazide
R" = OH (hydroxylamine), the product is an oxime

Scheme 4: Schiff base formation

Important derivatization reactions have been carried out with hydrazine. The most important substituted hydrazine is phenylhydrazine, which is prepared by the reduction of benzene diazonium chloride with stannous chloride and hydrochloric acid[37]. Hydrazines will react with carbonyl compounds to from hydrazones. An important reaction of phenylhydrazine is with the carbonyl functional group and simple sugars to give phenylhydrazones and osazones respectively. The crystalline properties of the osazones as well as their melting points are diagnostic of the types of sugars. Various derivatives of phenylhydrazine are used instead of phenylhydrazine. 2, 4-dinitrophenylhydrazine is a common reagent for the spot test of ketones and aldehydes. 2-nitrophenylhydrazine has been extensively used by Miwa, Yamamoto and Momose[38] to estimate carboxylic acids including salicylic acid produced in hydrolysis of aspirin. The intensely coloured hydrazides produced are often determined colorimetrically. Schiff base formation or hydrazine derivatization proceeds through a condensation reaction with the elimination of a mole of water. Majority of pharmaceuticals that have been assay *via* this technique come under skeletons possessing carbonyl functional groups or those with primary or secondary amino groups in which a suitable carbonyl group donor is used as reagent. Prominent carbonyl group donors have been *p*-dimethylaminobenzaldehyde[39], *p*-dimethylaminocinnamaldehyde[40]. Since the reaction involves condensation, many of such procedures are often carried out in acidified methanol and some require elevated temperatures. Similarly, dihydralazine has been determined in pharmaceuticals after derivatization with 2-hydroxy-1-naphthaldehyde[41].

4.0 Charge transfer complexation

Certain substances combine in a 1:1 molar ratio to form addition products. The molecular addition compound is held together by weak forces, such as Van der Waals. Poly nitro aromatic compounds, such as trinitrobenzene and picric acid, are well known for their ability to form charge – transfer complexes (Pi complexes).

Charge transfer complexes (electron donor - acceptor complexes) may be formed when an electron donor group is adjacent to an electron acceptor group. In this situation, experimental evidences suggest that the donor may transfer a portion of its charge to the acceptor. As a result, one compound becomes partially positively charged with respect to the other and a weak electrostatic bond is formed. Bond formation between the molecular pairs is brought about when filled π-orbitals (or nonbonding orbitals) in the donor overlap with depleted orbitals in the acceptor. The two new molecular orbitals formed are illustrated in the figure 2. The lower-energy molecular orbital (M.O.) for the complex is occupied in the ground state, and transitions from this M.O. to the new upper M.O. are responsible for the new absorption bands formed. The appearance of a new electronic absorption band, not attributable to either the donor or acceptor is often taken as an evidence for charge transfer complexing.

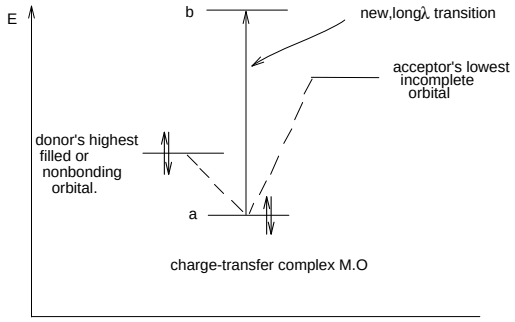
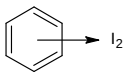


Figure 2: Electronic transitions for charge- transfer complexes. Donor and acceptor orbitals combine to form two orbitals (a

and b) for the complex. New electronic transitions for long λ are then possible between a and b.

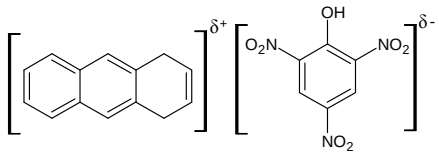
Since the formation of these complexes involves transfer of electronic charge from an 'electron-rich' molecule (a Lewis-base donor) to an 'electron-deficient' molecule (a Lewis-acid acceptor) they are called charge-transfer complexes [42].

Charge transfer donors and acceptors have been described and examples include: Benzene, Aminobenzene, Methoxybenzene, 1, 3, 5-trimethylbenzene, Anthracene (as charge- transfer donors) and Lewis acids and organic compounds such as Chloranilic Acid, Tetracyanoethylene (TCNE), Picric acid and Iodine (as charge – transfer acceptors). The structure of most charge-transfer complexes can be visualized as a face-to-face association on a 1:1 donor: acceptor basis: only thus, for example, can maximum overlap of aromatic π-orbitals take place. This kind of structure is difficult to draw, and most representations use one or other of the conventions shown below[42].



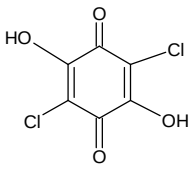
Donor Acceptor

or



Donor Acceptor

A wide range of reactions and reagents have been proposed for the analysis of pharmaceuticals by charge transfer complex formation. However, chloranilic acid has enjoyed the widest application as a π-electron acceptor while drugs possessing excess π-electrons or those with non-bonding *n*-electrons readily pair with it to form brilliantly coloured purple or pink adducts which are measured colorimetrically as a means of quantitation of the amount of analyte present.



Chloranilic acid (2, 5-dichloro-3, 6-dihydroxy-*p*-benzoquinone)

Chloranilic acid has been used for the spectrophotometric studies of some aminoheterocyclic donors[43], fifteen cephalosporins to give purple chromogens measured at 520 nm [44] and moclobemide as *n*-electron donor [45]. In all instances, chloranilic acid serves as a π-electron acceptor and acetonitrile is found to give the best result. The thermodynamic properties of the interactions of chloranilic and pyrimethamine-sulfadoxine combination drug have also been reported[46]. The interaction of chlorpromazine, promethazine, thioridiazine and prochlorperazine with chloranilic acid has been carried out and used as a means of quantitation of these phenothiazines[47] while charge transfer complexation with levofloxacin[48] and tetracyclines[49] have also been reported.

Some other charge transfer acceptors have found usefulness in the derivatization of pharmaceuticals. 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ); 7,7,8,8-tetracyanoquinodimethane (TCNQ), *p*-chloranil (CHL); Tetracyanoethylene (TCNE); quinhydrone; dichloroquinone-4-chloroimide (DCQ) and iodine have found relevance in this regard. DDQ has been used for the spectrophotometric determination of ciprofloxacin[50], terfenadine[51], nizatidine and ranitidine[52], rifampicin[53], naproxen and etodolac[54] and sodium flucloxacillin [55]. TCNE has been utilized for the analysis of norfloxacin[48], ciprofloxacin[50], colchicine[56], terfenadine[51], naproxen and etodolac[54]. On the other hand, CHL has been used extensively for the analysis of ciprofloxacin[50], rifampicin[53], diclofenac[57], nortriptyline[58], naproxen and etodolac[54] and amantadine[59]. TCNQ has found application for the spectrophotometric determination of trazodone, amineptine and amitriptyline[60], cinnarine, analgin and norfloxacin[61], sodium flucloxacillin[53], ciprofloxacin[50], terfenadine[51], rifampicin[53], Lisinopril[62], sodium flucloxacillin[55], cinnarizine, analgin and norfloxacin[61], trazodone, amineptine and amitriptyline[60]. Iodine has also found relevance as a σ-acceptor and more commonly adopted in chloroform as solvent, has been used for the analysis of amantadine HCl[59].

One clear fact emerging from the application of charge transfer complexation for the derivatization of drugs and chemicals is that more than one charge transfer acceptor is used for the particular drug or group of drugs. It is common to find chloranilic acid, p-chloranil, TCNQ, DDQ and TCNE being used for the analysis of similar groups of drugs.

Prominent assessments in the charge-transfer complexation reactions are selection of analytical wavelength, determination of mole or stoichiometric ratio and estimation of formation constant. The selection of analytical wavelength is commonly done by spectral overlay of the charge transfer reagent, the complex produced and the drug of interest. Since clearly defined violet, purple or pink colours are produced, the wavelength of the new absorbing species is often well into the colorimetric region without undue interference from either of the starting materials and with careful selection of the diluting solvents some degree of specificity is also afforded. This is illustrated in Figure 3 for the analysis of norfloxacin, analgin and cinnarizine using TCNQ.

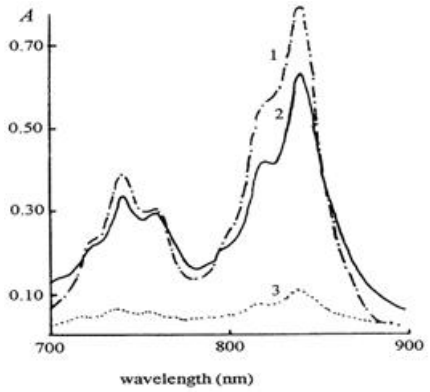


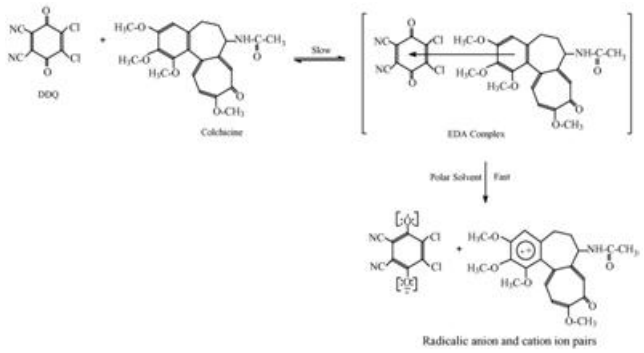
Figure 3: Absorption spectra: (1) TCNQ–norfloxacin against reagent blank, c (norfloxacin)-8.4x10^{-5} mol/l, (2) TCNQ–cinnarizine (analgin) against reagent blank, c(cinnarizine)-3.7x10^{-5} mol/l; c(analgin)-3.5x10^{-5} mol/l, (3) TCNQ–methanol against methanol, c(TCNQ)-4.00x10^{-3} mol/l.[61]

The stoichiometric ratio is usually carried out using Job's method of continuous variation whereby using equimolar concentrations of the reactants, the mole ratio that gives the highest difference in absorbance is selected as the optimal ratio for the charge transfer complex formation. More often than not, mole ratios of 1:1 are obtained. Since the formation of a new UV-absorbing species can proceed at different rates depending on the propensity of the reaction occurring, the formation constant is commonly calculated as an estimate of the spontaneity of formation of the complex. Benesi-Hildebrand equation has found particular usefulness in the estimation of the formation constant of molecular complexes and it is commonly utilized for this estimation.

$$\left(\frac{[A]o}{A} = \frac{1}{K_{CT}\varepsilon_{CT}} \cdot \frac{1}{[D]0} + \frac{1}{\varepsilon_{CT}}\right)$$

Where $[A]_0$ is the initial concentration of the acceptor (charge-transfer reagent), A is the absorbance of the charge transfer band, $[D]_0$ is the initial concentration of the donor (drug or chemical), K_{CT} is the formation constant of the new charge transfer band and ε_{CT} is the molar absorptivity. A plot of $[A]_0$ /A against $1/[D]_0$ will yield intercept as $1/\varepsilon$ and the slope as $1/K\,\varepsilon$ from where the formation constant and the molar absorptivity are obtained. The concentration of the acceptor is usually kept greater than the donor and fixed so that a wide concentration range could be adopted[63]. Some other physicochemical properties of the charge transfer bands are also estimated such as molar transition energy, oscillator strength, transition dipole, resonance energy, standard free energy and the ionization potential of the donor species; in order to establish the stability or otherwise of the formed complex between the drug and the charge transfer acceptor.

Charge transfer (CT) complexes result from a donor-acceptor mechanism of a Lewis acid-base reaction between two or more different constituents. CT complexes are associated with the appearance of new UV-VIS absorption bands[64]. CT complexes are sometimes produced as reaction intermediates[65-67] and most often they exist as stable donor-acceptor adducts[68-70]. One common mechanism for the CT complex formation is illustrated in scheme 5.



Scheme 5: Radical ion pair formation between DDQ and Colchicine [56]

5.0 Ion-pair analysis

Ion pair formation, initially investigated in physical chemistry was found extremely interesting for chemical analysis, including pharmaceutical analysis. Modern analytical methods (X-ray spectrometry, infrared spectrometry, UV-Vis spectrometry, resonance Rayleigh spectrometry) proved that the formation of ion pairs is a consequence of the electrostatic, hydrophobic and charge transfer interactions and allowed optimal experimental conditions setting for their formation[71].

Majority of the ion pair formations that have usefulness in pharmaceutical analysis are carried out with formation of ion pairs between drugs and dye molecules at pH values where the dyes can serve as charge donors. Subsequently, the ion pairs are extracted into organic solvents and spectra overlay is carried to determine the new wavelength of maximum absorption. Chloroform, being highly non-polar and immiscible with water has found the greatest relevance in the extraction of the ion-pair complexes. Because of this final extraction process, ion pair analysis in UV-VIS spectrophotometry is also referred to extractive spectrophotometric determination of drugs.

Bromocresol blue (BCB), bromocresol purple (BCP) and bromocresol green (BCG) have found great relevance as ion pair donors in most reported methods. These dyes have been used for the analysis of guanethidine sulphate (I), guanfacine hydrochloride (II), guanoclor sulphate (III), guanoxan sulphate (IV) and debrisoquine sulphate. The first method involves ion-pair formation of the selected compounds (I-V) with bromocresol purple at pH 3.8. The yellow ion pair is extracted with chloroform and the absorbance is measured at about 415 nm[72]. Two simple and sensitive extractive spectrophotometric methods have been developed for determination of zolmitriptan (ZTP) in tablets. These methods are based on the formation of yellow ion-pair complexes between ZTP and tropaeolin OO (TPOO) and bromothymol blue (BTB) in citrate-phosphate buffer of pH 4.0 and 6.0, respectively. The formed complexes were extracted with

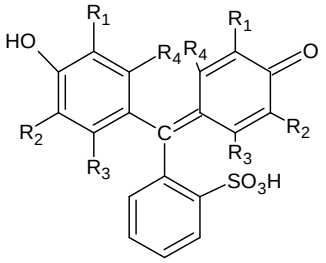
dichloromethane and measured at 411.5 and 410 nm for TPOO and BTB, respectively[73]. The first method was based on the formation of ion-pair complexes with the acidic sulfophthalein dyes bromocresol purple (BCP) and bromophenol blue (BPB) in pH 3.3 and 4.5 citrate-phosphate buffer, respectively. The formed complexes were extracted into dichloromethane, and their absorbance was measured at 403 and 410 nm for BCP and BPB, respectively[74]. A simple, accurate, rapid and sensitive spectrophotometric method has been developed for the assay of six phenothiazine derivatives in bulk drug and their pharmaceutical preparations. The method is based on ion-pair complex reaction of phenothiazines with bromocresol green in aqueous acidic buffer. The chromogen, being extractable with chloroform, could be measured quantitatively at 420 nm. All variables were studied in order to optimize the reaction conditions. The proposed method has been successfully applied to the analysis of the bulk drugs and their dosage forms, tablets and injections[75].

The following procedures illustrate the utilization of other dyes as donor groups in ion-pair analysis. Two simple and highly sensitive spectrophotometric methods were developed for the quantitative determination of the drug sildenafil citrate (SC), Viagra, in pure form and in pharmaceutical formulations, through ion-associate formation reactions (method A) with mono-chromotropic acid azo dyes, chromotrope 2B (I) and chromotrope 2R (II) and ion-pair reactions (method B) with bi-chromotropic acid azo dyes, 3-phenylazo-6-o-carboxyphenylazo-chromotropic acid (III), bis-3,6-(o-hydroxyphenylazo)-chromotropic acid (IV), bis-3,6-(p-N,N-dimethylphenylazo)-chromotropic acid (V) and 3-phenylazo-6-o-hydroxyphenylazo-chromotorpic acid (VI). The reaction products, extractable in methylene chloride, were quantitatively measured at 540, 520, 540, 570, 600 and 575 nm using reagents, I-VI, respectively. The reaction conditions were studied and optimized. Beer's plots were linear in the concentration ranges 3.3-87.0, 3.3-96.0, 5.0-115.0, 2.5-125.0, 8.3-166.7 and 0.8-15.0 µg mL^{-1} with corresponding molar absorptivities 1.02 x 10^4, 8.34 x 10^3, 6.86 x 10^3, 5.42 x 10^3, 3.35 x 10^3 and 2.32 x 10^4 L mol^{-1} cm^{-1} using reagents I-VI, respectively[76].

A simple, rapid and sensitive spectrophotometric method has been proposed for the assay of benzydamine HCl (BENZ), levamisole HCl (LEV) and mebeverine HCl (MBV) in bulk and pharmaceutical formulations. The method based on the reaction of the selected drugs with methyl orange (MO) in buffered aqueous solution at pH 3.6. The formed yellow ion-pair complexes were extracted with dichloromethane and measured quantitatively with maximum absorption at 422 nm[77].

Ulu and Aydogmus[78] reported the utilization of the bromophenol dyes and methyl orange for the assay of tianeptine by ion-pair analysis in acidic medium. The developed method involves formation of colored chloroform extractable ion-pair complexes of tianeptine with bromophenol blue (BPB), bromocresol green (BCG), bromothymol blue (BTB) and methyl orange (MO) in acidic medium. Beer's law is obeyed in the concentration ranges 3.0-12.0, 4.0-16.0, 4.0-14.0 and 2.0-10.0 µg mL^{-1} with BPB, BCG, BTB and MO, respectively. The detection limit of tianeptine was found to be 1.8 µg mL^{-1} for BPB, 2.0 for BCG, 2.0 µg mL^{-1} for BTB and 1.0 µg mL^{-1} for MO.

A simple, rapid and sensitive spectrophotometric method has been developed for the assay of ceterizine hydrochloride (CTZH) in bulk drug and its pharmaceutical preparations. This method is based on the ion-pair complex reaction between CTZH and Alizarin Red S in Clarks-Lubs buffer. The chromogen being extractable with chloroform could be measured quantitatively at 440 nm. All variables were studied to optimise the reaction conditions. Regression analysis of Beer's Law plot showed good correlation in the concentration range 2.5-22 µg/ mL[79]. Alizarin red S has also been used for the ion-pair analysis of H_1 antagonists, chlorphenoxamine hydrochloride (CPX), diphenhydramine hydrochloride (DPH) and clemastine (CMT) in bulk and in their pharmaceutical formulations. The method is based on the formation of an ion-association complex with alizarin red S as chromogenic reagents in acidic medium (Method B), which is extracted into chloroform. The complexes have a maximum absorbance at 425 and 426nm for (DPH or CMT) and CPX, respectively[80].



Dye	BTB	TB	BPB	BCG	PR	CR
R_1	$CH(CH_3)_2$	$CH(CH_3)_2$	Br	Br	H	H
R_2	Br	H	Br	Br	H	CH_3
R_3	CH_3	CH_3	H	H	H	H
R_4	H	H	H	CH_3	H	H

BTB=Bromothymol blue, TB=Thymol blue, BPB=Bromophenol blue, PR=Phenol red, CR=Cresol red

6.0 Complexation reactions

Complexation reactions have been adopted as an age-long approach for the analysis of metals and metalloids in water, pharmaceutical preparations and other matrices. The procedure usually involves the selection of appropriate complexing agents, controlling the pH and appropriately selecting the temperature and solvents required. Once the metal ions bind the ligand specific colour changes are observed which can be quantitated as a function of the amount of metal ions present. This procedure is also utilized for the gravimetric analysis of metal ions. In recent times, the ability of some organic pharmaceuticals to serve as ligands has also been utilized for the estimation of these drugs. However, few metal ions show such strong colours, particularly at low

concentrations. Fortunately many highly colored complexes can be formed from metal ions and organic or inorganic complexing agents. These complexes are the result of the interaction of a Lewis acid (the metal ion) and a Lewis base (the complexing agent). The ideal colour-forming reagent should be stable and selective (even specific) and react rapidly to form soluble, highly colored complexes. The colored complex should have a high absorptivity and be free from variations in colour due to minor changes in pH or temperature.

The application of colorimetric reagents is not a new technique but dates back nearly two thousand years. Around 60 A.D. Pliny the Elder in his "Natural History" recommended the use of nutgall as a reagent for the determination of iron in verdigris, which is a green pigment. Nutgall contains about 65-70% tannic acid which when combined with iron leads to the formation of a black iron tannate complex. In general organic colorimetric reagents are considerably more sensitive than are inorganic ones. They give more intense colours and are therefore frequently used for trace analyses. With many organic reagents, it is possible to determine concentrations at the ppm level. 2,2'-Bipyridyl (bipy), gfw = 156.20, forms an intensely red complex with iron(II) which may be exploited to determine iron concentrations in the ppm range

In a combination of oxidation-reduction mechanisms with complexation reaction lansoprazole (LPZ) has been determined in bulk drug and in capsule formulation. The methods are based on the oxidation of lansoprazole by *in situ* generated bromine followed by determination of unreacted bromine by two different reaction schemes. In one procedure (method A), the residual bromine is treated with excess of iron (II), and the resulting iron (III) is complexed with thiocyanate and measured at 470 nm. The second approach (method B) involves treating the unreacted bromine with a measured excess of iron (II) and remaining iron (II) is complexed with orthophenanthroline at a raised pH, and measured at 510 nm. In both methods, the amount of bromine reacted corresponds to the amount of LPZ[81].

A new method for the determination of cerium subgroup rare earths was studied and reported using Dibromo-*p*-methyl-chlorosulfonazo (DBMCSA) as a new ligand. It was found that cerium subgroup rare earth elements react with DBMCSA in 0.6 mol/L hydrochloric acid medium to form stable blue complexes. The absorbances of equal amounts of cerium subgroup rare earths are close to each other at their maximum adsorptive wavelength (641 nm). Beer's law is obeyed for 0-20 µg of rare earths in 25 mL of solution. The method has been applied to the determination of the total amount of cerium subgroup rare earths in steel and cast iron samples with satisfactory results[82].

A simple, sensitive and accurate spectrophotometric method of analysis of ceftriaxone, cefotaxime and cefuroxime in pharmaceutical dosage forms has been developed and validated. The method is based on the formation of Prussian Blue (PB) complex. The reaction between the acidic hydrolysis product of the antibiotics (T = 70°C) with the mixture of Fe^{3+} and hexacyanoferate (III) ions was evaluated for the spectrophotometric determination of the antibiotics. The maximum absorbance of the coloured complex occurred at λ = 700 nm and the molar absorptivity is 3.0 x 10^4 $L.mol^{-1}$ cm^{-1}. Reaction conditions have been optimized to obtain PB complex of high sensitivity and longer stability. Under optimum conditions the absorbance of the PB complex were found to increase linearly with increase in concentrations of ceftriaxone, cefotaxime and cefuroxime, which corroborated with the correlation coefficient values. The linear range of the calibration graph was 2 - 20 µg/mL for ceftriaxone and cefotaxime and 2 - 18 µg/mL for cefuroxine [83].

Traces amount of fluoride have also been determined using Al-Xylenol reagent as a complexing agent. The decolourising effect of fluoride on the metal-dye complex was adopted as a means of quantitation of fluoride in water samples[84].

A simple and sensitive spectrophotometric method has been developed for the determination of thioctic acid in pharmaceutical preparations. The proposed method is based upon the formation of a complex with palladium(II) in acetate buffer of pH 4.8 with an absorption maximum at 318 nm. The absorbance obeyed Beer's law over the range of 2-20 µg mL^{-1} with a minimum detection limit of 0.15 µg mL^{-1} and molar absorptivity (ε) of 7 x 10^3 L $mol^{-1}.cm^{-1}$. The different experimental parameters affecting the development and stability of the colour were carefully studied and optimized. The proposed method was successfully applied to the analysis of commercial tablets and ampoules[85].

A new, simple, precise, rapid and low-cost spectrophotometric method for methyldopa determination in pharmaceutical preparations is described. This method is based on the complexation reaction of methyldopa with molybdate. Absorbance of the resulting yellow coloured product is measured at 410 nm. Beer's Law is obeyed in a concentration range of 50 – 200 µg mL^{-1} methyldopa with an excellent correlation coefficient (r = 0.9999). No interference was observed from common excipients in formulations. The results show a simple, accurate, fast and readily applied method to the determination of methyldopa in pharmaceutical products [86].

A simple, sensitive, and accurate spectrophotometric method has been developed for the assay of furosemide (FUR), which is based on the complexation of the drug with copper(II) at pH 3.2 using Mclivaine buffer solution to produce a green adduct. The latter has maximum absorbance at 790 nm and obeys Beer's law within the concentration range 5–30 µg/mL. Regression analysis of the calibration data showed a good correlation coefficient (r = 0.9997) with minimum detection limit of 0.23 µg/mL.

The proposed procedure has been successfully applied to the determination of this drug in tablets. In addition, the spectral data and stability constant for the mononuclear copper(II) complex of furosemide ($CuFUR_2(MeOH)_2$) are reported [87].

A spectrophotometric method has been developed for the determination of amlodipine besylate in pure form and in pharmaceutical preparations. The method is based on the reaction of the primary amino group of the drug with ninhydrin in N,N'-dimethylformamide (DMF) medium producing a coloured complex which absorbs maximally at 595 nm. Beer's law is obeyed in the concentration range of 10-60 µg mL^{-1} with RSD of 0.66% and molar absorptivity of 6.52 x 10^3 L mol^{-1} cm^{-1}. All variables were studied in order to optimize the reaction conditions. The proposed method has been applied successfully to the analysis of the bulk drug and its dosage forms. No interference was observed from common pharmaceutical adjuvants. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision[88].

A novel simple kinetic spectrophotometric method for the determination of N-acetyl-L-cysteine (NAC) has been developed and validated. The proposed method is based on a coupled redox-complexation reaction, the first step of which is the reduction of Fe^{3+} by NAC; the second one includes the complexation of Fe^{2+}, resulting from the preceding redox reaction, with 2,4,6-trypyridyl-s-triazine (TPTZ). The stable Fe $(TPTZ)^{(2)(2+)}$ complex exhibits an absorption maximum at λ = 593 nm. The initial rate and fixed-time (at 5 min) methods were utilized for constructing calibration graphs. The graphs were linear in concentration ranges from 4.0 x $10^{-6}$to 1.0 x 10^{-4} mol L^{-1} for the initial rate method and 1.0 x 10^{-6} to 1.0 x 10^{-4}mol L^{-1} for the fixed-time method, with detection limits of 1.0 x 10^{-6} and 1.7 x 10^{-7} mol L^{-1}, respectively. The proposed methods were successfully applied for the determination of NAC in its commercial pharmaceutical formulations[89].

An indirect spectrophotometric determination of some biologically important phenothiazines has also been carried out using the complexation mechanism. The drugs; chlorpromazine, promethazine, trifluoperazine, prochlorperazine and fluphenazine were reacted with a fixed amount of dichromate in acidic conditions. The unreacted dichromate was then determined by treating with Iron (II) and *ortho*-phenanthroline at a raised pH and measuring the absorbance at 510 nm[90].

7.0 Oxidation-reduction reactions

Some derivatization techniques have also been reported based on the ability of drug molecules to serve as either oxidant or reductant in a given reaction and to generate coloured substances that can be used for quantitative estimation. In other instances, prior conversions of drugs to species that can be involved in oxidation-reduction reactions are also carried out.

Amantadine HCl was determined by oxidation of the drug by ammonium molybdate. Amantadine HCl was subjected to the oxidation by ammonium molybdate, Mo(VI), which was consequently reduced to the corresponding Mo(V) ions that are blue colored of λ_{max} at 780 nm[91].

A simple and cost effective spectrophotometric method is described for the determination of torsemide in pure form and in pharmaceutical formulations. The method is based on the formation of blue colored chromogen when the drug reacts with Folin-Ciocalteu (F-C) reagent in alkaline medium. The colored species has an absorption maximum at 760 nm and obeys beer's law in the concentration range 30 – 150 µg mL^{-1}. The absorbance was found to increase linearly with increasing concentration of TSM, which is corroborated by the calculated correlation coefficient value of 0.9999 (n=8). The apparent molar absorptivity and Sandell sensitivity were 1.896 x10^3 L mol^{-1} cm^{-1} and 0.183 µg cm^{-2}, respectively. The slope and intercept of the equation of the regression line are 5.4 x10^{-3} and 1.00 x10^{-4} respectively. The limit of detection was 0.94. This procedure adopts the well known reduction reaction involving Folin-Ciocalteu (F-C) reagent to generate a blue chromogen[92]. Likewise, new colorimetric methods for the estimation of aceclofenac and indapamide from their respective tablet formulation were developed based on the formation of blue and green colored chromogen with Folin-Ciocalteu (FC) reagent[93].

Three simple, sensitive and reproducible spectrophotometric assays (A-C) for the determination of etodolac in pure form and in pharmaceutical formulations have been reported. Methods A and B are based on the oxidation of etodolac by Fe^{3+} in the presence of *o*-phenanthroline (o-phen) or bipyridyl (bipy). The formation of the tris-complex on reaction with Fe^{3+}-*o*-phen and/or Fe^{3+}-bipy mixtures in acetate buffer solution at optimum pH was demonstrated at 510 and 520 nm with *o*-phen and bipy. Method C is based on the oxidation of etodolac by Fe^{3+} in acidic medium, and the subsequent interaction of iron(II) with ferricyanide to form Prussian blue, with the product exhibiting an absorption maximum at 726 nm. The concentration ranges are 0.5–8, 1.0–10 and 2– 18 µg mL^{-1} respectively for methods A, B and C. For more accurate analysis, Ringbom optimum concentration ranges were calculated, in addition to molar absorptivity, Sandell sensitivity, detection and quantification limits[94].

Four sensitive and rapid methods for the determination of stavudine (STV) in bulk drug and in dosage forms were developed and optimized. 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. Spectrophotometric methods involve the addition of a measured excess of bromate-bromide in HCl medium and subsequent estimation of the residual bromine by reacting with a fixed amount of methyl orange, indigo

carmine or thymol blue followed by measurement of absorbance at 520 nm (method A), 610 nm (method B) or 550 nm (method C). In all the methods, the amount of bromate reacted corresponds to the amount of STV. Calculations in titrimetry were based on a 1:0.666 (STV:$KBrO_3$) stoichiometry and the method was found to be applicable over 3.5-10 mg range. A linear increase in absorbance with concentration of STV was observed in the spectrophotometric methods, and the Beer's law was obeyed over the concentration ranges 0.125-1.75, 1-10 and 1-9.0 µg mL^{-1} STV for method A, method B and method C, respectively. The methods when applied to the determination of STV in tablets and capsules were found to give satisfactory results[95].

N-haloimides have been used as effective oxidizing/brominating agents for the spectrophotometric determination of many pharmaceutical compounds[96,97]. N-bromosuccinimide (NBS), being the most versatile, is the most commonly used haloimide[98,99]. The analysis involving NBS was based on direct measurement of the chromogenic derivative of the drug, or indirectly by measuring the remaining NBS with color-producing reagents susceptible to oxidation or bromination with NBS. It was reported that p-aminophenol (PAP) is easily susceptible to oxidation with NBS and gives a violet chromogenic product of lmax at 552 nm[100]. One titrimetric and two spectrophotometric methods which are simple, sensitive and rapid are described for the assay of salbutamol sulphate (SBS) in bulk drug and in tablet dosage forms using N-bromosuccinimide (NBS) and two dyes, rhodamine-B and methylene blue, as reagents. In titrimetry, aqueous solution of salbutamol sulphate is treated with a measured excess of NBS in acetic acid medium and after the oxidation of SBS is complete, the unreacted oxidant is determined iodometrically. Spectrophotometric methods entail addition of a known excess of NBS in acid medium followed by the determination of residual oxidant by reacting with a fixed amount of either rhodamine B and measuring the absorbance at 555 nm (method A) or methylene blue and measuring the absorbance at 665 nm (method B). In all methods, the amount of NBS reacting corresponds to the amount of SBS content. Titrimetric method is applicable over 1.74×10^{-4} - 8.68×10^{-4} mol L^{-1} range and the reaction stoichiometry is found to be 1:6 (SBS:NBS). In spectrophotometric methods, the absorbance is found to increase linearly with the concentration of SBS, which is corroborated by the correlation of coefficients of 0.9993 and 0.9988 for method A and method B, respectively. The systems obey Beer's law for 0.25-1.75 µg mL^{-1} (method A) and 0.5-5.0 µg mL^{-1} (method B). The calculated apparent molar absorptivity values were found to be 2.10×10^5 and 6.16×10^4 L mol^{-1} cm^{-1}, for method A and method B, respectively. The limits of detection and quantification are also reported for both spectrophotometric methods. Intra-day and inter-day precision and accuracy for the developed methods were evaluated. The methods were successfully applied to the assay of SBS in tablet and capsule formulations and the results were statistically compared with those of a reference method. No interference was observed from common tablet adjuvants. The accuracy and reliability of the methods were further ascertained by recovery experiments *via* the standard-addition technique[101].

Ceric ammonium sulphate has also found relevance in oxidation-reduction spectrophotometry. It is commonly adopted for the oxidation of drugs that possess reducible moieties and in turn it gets oxidised to the cerate ion which has an intense yellow colour. In some determinations, the amount of residual cerium sulphate is determined by further reactions with such compounds as indigo carmine, methyl orange or *p*-dimethylaminobenzaldehyde. Two spectrophotometric methods are proposed for the assay of lansoprazole (LPZ) in bulk drug and in dosage forms using ceric ammonium sulphate (CAS) and two dyes, methyl orange and indigo carmine, as reagents. The methods involve addition of a known excess of CAS to LPZ in acid medium, followed by determination of residual CAS by reacting with a fixed amount of either methyl orange, measuring the absorbance at 520 nm (method A), or indigo carmine, measuring the absorbance at 610 nm (method B). In both methods, the amount of CAS reacted corresponds to the amount of LPZ and the measured absorbance was found to increase linearly with the concentration of LPZ, which is corroborated by the correlation coefficients of 0.9979 and 0.9954 for methods A and B, respectively. The systems obey Beer's law for 0.5-7.0 µg mL^{-1} and 0.25-3.0 µg mL^{-1} for methods A and B, respectively. The apparent molar absorptivities were calculated to be 3.0×10^4 and 4.4×10^4 L mol^{-1} cm^{-1} for methods A and B, respectively. The limits of detection (LOD) and quantification (LOQ) were calculated to be 0.08 and 0.25 µg mL^{-1} for method A, and 0.09 and 0.27 µg mL^{-1} for method B, respectively. The intra-day and inter-day precision and accuracy of the methods were evaluated according to the current ICH guidelines. Both methods were of comparable accuracy (er < or = 2 %). Also, both methods are equally precise as shown by the relative standard deviation values < 1.5%. No interference was observed from common pharmaceutical adjuvants. The accuracy of the methods was further ascertained by performing recovery studies using the standard addition method. The methods were successfully applied to the assay of LPZ in capsule preparations and the results were statistically compared with those of the literature UV-spectrophotometric method by applying Student's t-test and F-test[102].

In dilute sulphuric acid medium formaldehyde is oxidised to formic acid. Formic acid does not reduce any ceric sulphate under the above conditions. However formic acid is oxidised quantitatively to carbon dioxide and water by cerate-chromate reagent. Based on this principle a method has been worked out for the estimation of a mixture of formaldehyde and formic acid. Moreover it has been shown that the cerate-chromate reagent has no effect on acetic acid. Formic acid and acetic acid can be

titrated against standard alkali and the total determined as acid. Based on these two different reactions a method has been worked out for the estimation of mixtures of formic and acetic acids[103].

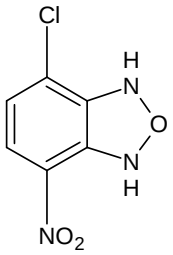
The quinolones and other drugs have been particularly analysed using oxidation-reduction spectrophotometry. Basavaiah *et al* reported the quantitation of ciprofloxacin based on oxidation with cerium (IV) sulphate and then determining excess oxidant with indigo carmine or methyl orange[104]. However, Adegoke and Balogun reported the spectrophotometric determination of three quinolones (ciprofloxacin, sparfloxacin and perfloxacin) using Ce(IV) with determination of excess oxidant by a reddish-brown colour formation with *p*-dimethylaminobenzaldehyde (DMAB)[105]. Ce(IV)/DMAB system has also been adopted by for the spectrophotometric determination of H_2-receptor antagonists through oxidation[106]. Cerium sulphate has also been used for the spectrophotometric determination of ciprofloxacin with extraction into chloroform[107].

A simple and sensitive kinetic-spectrophotometric method was developed for the determination of ultra trace amount of formaldehyde in food samples. The method was based on the oxidation of rhodamine B (RhB) by potassium bromate in sulfuric acid medium (formaldehyde as catalyst). The reaction was monitored by measuring the decrease in absorbance of the dye at 515 nm after 6 min. The developed method allowed the determination of formaldehyde in the range of 10–100 µg L^{-1} with good precision, accuracy and the detection limit was down to 2.90 µg L^{-1}. The relative standard deviations for the determination of 10 and 60 µg L^{-1} of formaldehyde were 3.0% and 1.9% (n = 10), respectively. The method was found to be sensitive, selective and was applied to the determination of formaldehyde in foods with satisfactory results[108].

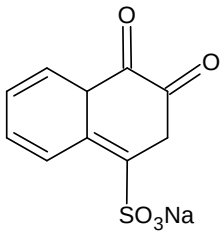
8.0 Miscellaneous methods

Some other reagents have been used for the determination of a wide range of pharmaceuticals and this section presents an overview of some of these reagents.

4-Chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) is an activated halide that has been used for the colorimetric determination of some primary and secondary amines. NBD-Cl has been reported as fluorogenic reagent for determination of amines [109] and for spectrophotometric determination of many compounds such as; vigabatrin[110], paroxetine[111], superoxide ion[112], lisinopril[113], dothiepin hydrochloride[114], β-blockers[115], tramadol hydrochloride[116], Salbutamol[117] and certain cephalosporins[118]. Thiol compounds have been reported to form intensely colored products in an alkaline medium with NBD-Cl which could be used for their colorimetric determination[119]. NBD-Cl has also been adopted for the colorimetric derivatization of acetylcysteine and captopril[120], both which contains thiol group.



NBD-Cl



NQS

Sodium 1,2-naphthoquinone-4-sulphonic acid (NQS) has been used as a chromogenic reagent for the spectrophotometric determination of many pharmaceutical amines where a condensation reaction occurs. Some of the compounds determined by NQS are dopamine HCl[121], glycine and lysine[122], aminomethylbenzoic acid[123], trimetazine dihydrochloride[124], ampicillin sodium[125] and paroxetine[126].

Another popular derivatization reagent is sodium nitroprusside (SNP). SNP was first described by Playfair[127] in 1849. The compound has attracted considerable interest at various periods in chemical history. The pharmaceutical interest in SNP is due to its applications as an analytical reagent as well as a strong hypotensive agent. It has been known for many years as a color-forming reagent with thiols and some other sulphur-containing compounds or ions [128]. It has been used as a valuable reagent for the detection and determination of wide variety of nucleophilic agents such as primary or secondary aliphatic amines, aldoximes, ketones, nitriles, phenols, pyrroles, quinones, thiols, thioureas and uracils [129]. An intensive review for the physical, chemical and pharmacological properties of SNP as well as its applications as an analytical reagent was provided by Leeuwenkamp et al. Different techniques adapted SNP as an analytical reagent in their applications. These techniques include HPLC, flow injection analysis and spectrophotometry. SNP has also been reported for the assay of rosoxacin antibiotics [130].

3-methyl-2-benzothiazolinone hydrazine (MBTH) HCl has also been used for the chemical derivatization of pharmaceuticals. It has been successfully applied to the determination of ceftazidime [131] and oxcarbazepine [132] with good accuracy, precision and excellent calibration data.

9.0 Selection of analytical wavelength and validation

In almost all of the methods reported for the spectrophotometric determination of pharmaceuticals following chemical derivatization, selection of analytical wavelength is commonly done by overlaying the absorption spectra of resulting derivatized compound over that of the reagent and the drug being derivatized. Working wavelength is selected at the region where there is optimal difference in the absorptivity of the derivatives compared to the starting materials. In some instances, the working wavelength may not correspond to the wavelength of maximum absorption (λ_{max}) as interference may be present at this wavelength. In determinations

where pronounced bathochromic shift is observed following derivatization, assessment of concentration of derivatives is done at the λ_{max} without the possibility of interference. This mode of determination occurs readily in majority of the charge-transfer complexation reactions and in some azo dye derivatization procedures. The observance of pronounced hyperchromic shift following derivatization often preclude the utilization of λ_{max} as the working wavelength and hence optimal difference in absorptivity is determined with a view to affording some measure of selectivity and lack of interference with determination.

Reported validation parameters for derivatization procedures

According to the ICH guidelines, there are minimum parameters to be satisfied before a derivatization procedure can qualify to be adopted as an alternative method. Analytical methods should be characterised and documented fully, and their reliability in the specified area of application demonstrated before they are brought into use. Such method-validation ensures that methods are under statistical control and are fit for their intended purpose. Validation should cover all stages through sample selection and preparation, analyte recovery, calibration of equipment, the analysis protocol and the assessment, interpretation and reporting of results. Validation of methods for the quantitative analysis of drugs involves determining, as a minimum, their selectivity, limit of detection, limit of quantification, linearity, working range, accuracy, precision and ruggedness under the conditions and with the typical sample matrices that will be met in practice. For qualitative analysis, usually only the selectivity, limit of detection and ruggedness are important. Where there is a predefined threshold concentration for reported results, the accuracy and precision should be determined at the threshold level. For methods that are to be used by more than one laboratory, each laboratory should verify the method, and the inter-laboratory variation should be determined. These data should be used to define how the performance of the method is to be monitored through QC and to specify what performance is fit for purpose. If it is necessary to compare the results with those from other methods, the compatibility of data from the different methods should also form part of the validation. Any subsequent change at any stage of the method, or in the sample matrix or concentration range, will require revalidation of the method. The extent and requirements of revalidation will depend on what changes have been made. Where a laboratory adopts an already validated method, it should demonstrate that the performance characteristics it can achieve are fit for the intended purpose of the method. Particularly important in this respect are selectivity (if the sample matrix is different), limit of detection, accuracy and precision [133,134].

Apart from these minimum parameters, recent literature discusses the analytical signal stability of the derivatized procedures. The assessment of analytical signal stability involves measuring the absorbance of the derivatives over a given period of time at timed intervals and different exposures to diffuse light with a view to making sound declaration about the applicability of the method. Since most derivatization methods lead to formation of coloured products assessment of stability of the signal in diffuse light becomes critical and majority of the procedures are often required to be carried out protected from light or samples wrapped away to avoid photodecomposition.

It is also a requirement that newly described procedures are compared statistically with established method preferably those recommended by the pharmacopoeias. However, a reference method is also adopted for comparison in some instances. All the reported procedures usually adopt student t-test and F-ratio tests for comparing the accuracy and precision of the developed method with official method respectively at a pre-determined probability level of 0.05. When the calculated p> 0.05 in both instances, no statistical significance is reported and the methods can be interchanged. When more than two developed procedures are compared, analysis of variance (ANOVA) test is carried out.

OVERVIEW AND FUTURE PROSPECTS

Chemical literature has witnessed a profound increase in the number of derivatization procedures over the years and more reagents are being introduced regularly for the assessment of pharmaceuticals. The reason is not far-fetched as most of the developed methods are preferably cheaper, simpler and adopt more ready instrumentation compared to sophisticated techniques involving HPLC, GC, Capillary electrophoresis, bioassays and other techniques.

Apart from the well-recognised reactions involving azo dye formation and Schiff base synthesis, more methodologies have been described over the years involving such techniques as complexation reactions, acid-dye or ion-pair techniques, acid-base reactions and oxidation-reduction procedures. In all of these categories, new reagents are commonly reported or new applications for old reagents are described.

One great advantage of chemical derivatization methodologies is the improved sensitivity, selectivity and wide applicability of the procedures. Since many of the methodologies involve well-defined reactions, the analyte levels to be determined often gets into micro-grams levels. Sandell's sensitivity which is defined as µg/mL of analyte per 0.001A is commonly used to characterize the sensitivity of the procedures. Low Sandell's sensitivities have been reported in majority of the techniques thus increasing the tendency for better application. Since the reagent used for chemical derivatization often pick up analytes based on well-defined chemical principles, the reactions can be highly selective and more also it can be made even more selective by change of solvent, pH or other relevant reaction conditions. Wide applicability is

another wonderful advantage of chemical derivatization methodologies in that some of the reactions can be readily modified to suite certain functional groups. For instance, majority of reactions undergone by amines can also be applied to nitro derivatives following a single step of reduction process.

As relevant as these derivatization methodologies are, some drawbacks are commonly observed. One major disadvantage with derivatization methodologies is the stability of the reagents used and that of the adduct or derivatives formed. Most the reagents adopted from oxidation-reduction reactions to complexation and compound formations are required to be stored under strictly defined conditions. And many a times the derivatives resulting are also required to be determined within a specified period of time in order to afford reproducibility of results. However, when appropriate precautions regarding storage are taken this demerit is overcome. Evaluation of analytical signal stability is common procedure in most derivatization methodologies with absorbance values taken at pre-determined timed intervals under diffuse light and when samples are wrapped. A nearly unchanging absorbance pattern over the time interval gives a picture of the stability of the formed derivative.

The other demerit is the ability of some of the reagents to give multiple products in reactions. For instance, in diazo coupling reactions used for azo dye derivatization possibility of forming *ortho*- and *para*-substituted products occurs with the tendency that the absorption being measured is due to multiple products rather a single compound. One way of circumventing this is to carefully select the reaction conditions that favour the formation of one positional isomer relative to the other in order to accurately determine the compound of interest. Prior thin layer chromatographic analysis of reaction mixture before spectrophotometric determination has been found to also control the tendency for determining multiple products rather the compound of interest. In some developed methods, separation of major products from the minor products using solid phase cartridges have been used to improve selectivity of reaction.

Majority of the reagents may not also be particularly selective for the compounds of interest with the possibility that similar compounds to be derivatized when present may yield compound with the same or closely related spectral characteristics. For instance, majority of reagents will give close λ_{max} for congeners and also similar patterns may be observed for a drug and its degradation product. Once again prior TLC analysis will yield information if such interferents are present and thus appropriate precautions can be taken to ensure selectivity.

Interference from excipients used in the manufacture of various dosage forms is also another major consideration in the design and execution of a given derivatization methodology. In most of the reported methods, an investigation of the level of interaction of the various excipients with the reagent under the condition of the experiments is expected to be part of the method validation. The practice is to study the recovery of quality control samples from the matrices containing common excipients and then make a pronouncement as to which excipient is interfering or not.

The use of organic solvents in the final stages prior to spectrophotometric analysis of derivatized compounds has often been quoted as a disadvantage by most authors as the disadvantage justifying the design and development of newer derivatization methodologies. It is often believed that extraction of the coloured substances produced from the chemical derivatization step into an organic solvent layer will improve selectivity and indeed it does, but the need to control the temperature of the environment and minimise undue exposure to the atmosphere cannot be overemphasised if accurate measurements will be obtained. Still related to the use of organic solvents is current concern about the environmental safety of most of the reagents adopted in derivatization procedures. However, in laboratories with appropriate waste disposal facilities, the use of organic solvents will not pose serious environmental hazards.

Some alternative methods have also criticized the use of buffer systems and aqueous media in the sample preparation stages. While it is often assumed that buffer solutions and their preparation might prolong the method application stages buffers have been used to increase the selectivity of some procedures since the reagent and the derivatized compounds can be made to give specific light absorption removed from interference substances.

One major criticism once again with most derivatization techniques is the use of multi-step stages in the development and application of the procedures. Thus for some reactions that involve reduction, diazotization and coupling reaction in azo dye derivatization methodologies, a three-step process is designed and developed and some others could involve just two and even four steps. While all these steps may improve selectivity and contribute to sensitivity of the procedure, multi-step stages increase the possibilities of committing errors, especially human errors.

The relevance of derivatization methodologies will continue to increase as the years go by because of the increased cost of analysis of pharmaceuticals when sophisticated techniques are adopted. The recent pharmacopoeias however is filled with methodologies involving some of these expensive techniques with the derivatization procedures now reserved for simple test tube reactions for the purpose of identification. However, it is anticipated that derivatization methodologies will continue to be relevant in poor-resource economies for the quality control of majority of the pharmaceuticals that circulate in these countries. The likelihood of introduction of newer reagents in the near future cannot be ruled out.

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