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Review Article

UTILIZATION OF EOSIN DYE AS AN ION PAIRING AGENT FOR DETERMINATION OF PHARMACEUTICALS: A BRIEF REVIEW

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ABSTRACT

Globally, dyes are widely used in the pharmaceutical, food, textile, cosmetics, plastics, leather, paint, ink and paper industries. Eosin is an acidic orange-pink dye and has very strong staining properties. Haematoxylin and eosin Y (H and E) combination is the most common staining and primary diagnostic technique in histo-pathological laboratories. This review mainly discussed the utility of eosin dye in quality control laboratories as an ion pairing agent for drug analysis. Eosin Y is one the most common ion pairing agent and its mono and di anionic forms are capable of interacting with many drug molecules to form colored/fluorescent binary or ternary complexes that can be analyzed with or without extraction by spectrofluorimetry and/or spectrophotometry. Quenching fluorescence and advantages of spectrofluorimetry over spectrophotometry were also discussed. Fluorescence detection greatly enhances the sensitivity and providing a sensitive and relatively inexpensive instrumental method of analysis using eosin Y for various important drugs in pure, commercial dosage forms and biological fluids.

Keywords: Eosin Y, Ion pairing agent, Binary and ternary complexes, Commercial dosage forms

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INTRODUCTION

Dyes are natural or synthetic colored organic substances which have the affinity to impart color to various substrates by absorbing into the substrate. In general, the dye molecules are chemically bonded to the surface and become a part of the material on which it is applied. Dyes are widely used in the pharmaceutical, food, textile, cosmetics, plastics, leather, paint, ink and paper industries [1]. A class of dye called xanthene is classified into three subgroups. One of the subgroups is called fluorone dyes that include fluorescein, erythrosine and rhodamine.

Eosin dyes are bromine derivative of fluorescein which has two very closely related dyes commonly known as Eosin yellowish (Eosin Y) and Eosin bluish (Eosin B) as shown in fig. 1. Eosin Y is a tetrabromo derivative whereas eosin B is a dibromo dinitro derivative of fluorescein.

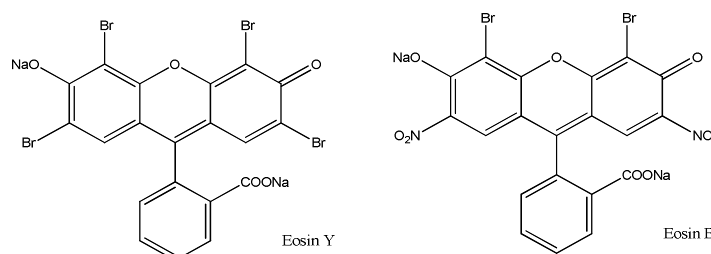

Fig. 1: Structure of eosins

Eosin Y is chemically known as disodium 2-(2, 4, 5, 7-tetrabromo-6-oxido-3-oxo-3H-xanthen-9-yl) benzoate have a molecular formula ($C_{20}H_6Br_4Na_2O_5$) and molar mass 691.85. Fluorescein in the eosin Y molecule called fluorochrome exists in two forms; one is the more stable quinoid form which is colored and fluorescent while the other one is lactone form which is colorless and non-fluorescent presented as shown A and B respectively, in fig. 2 [2]. Disodium salt of eosin Y can be readily converted into free acid in which the free carboxylic acid group (quinoid form) exists in equilibrium with its lactone form. In a weakly acidic medium, ionization of eosin Y may take place either by dissociation of hydroxyl or carboxylic group.

It was reported that hydroxyl group dissociates easily compare to the carboxylic acid group. In solution, eosin Y can exist in three different forms as neutral (H_2R), monoanionic (HR^-) and di anionic (R^{2-}) forms, where R denotes the anionic part of the eosin Y as explained in fig. 3 [3-4]. The ionization constants (pKa_1 and pKa_2) of eosin Y were reported as 2.9 and 4.5 [5].

Eosin Y is a Biological Stain Commission certified dye. The HandE stains the most common and primary diagnostic technique in histo-pathological laboratories to stain cells and cytoplasm, collagen and muscle fibers. H and E staining method was also used in structure determination of grasshopper and mammalian testis as well as supporting structure determination of destruction of dental tissues [6]. Eosin was utilized as a fluorescent indicator in acid-base titration for the determination of vitamins (B_1 and B_2) in food [7]. Due to its strong staining properties, it has been used in various industrial applications such as color filter, liquid crystal display, paper inks, photographic materials, laundry detergent, cosmetics, pencil lead, pigments, varnish and textiles. It is also used in medicine as an important radioactive tracer.

Literature surveys reported that the mono or di anionic forms of eosin Y are capable of interacting with a cationic form of the drug molecules by the electrostatic interaction and hydrophobic forces and forms either binary complex with drug molecule or a ternary complex with drug molecule and a metal [8].

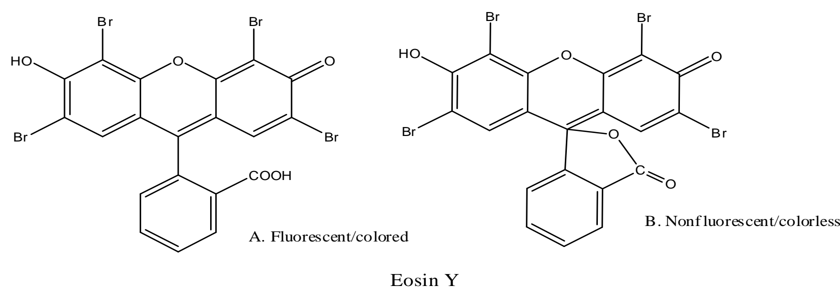

Fig. 2: Different forms of fluorochrome (Eosin Y)

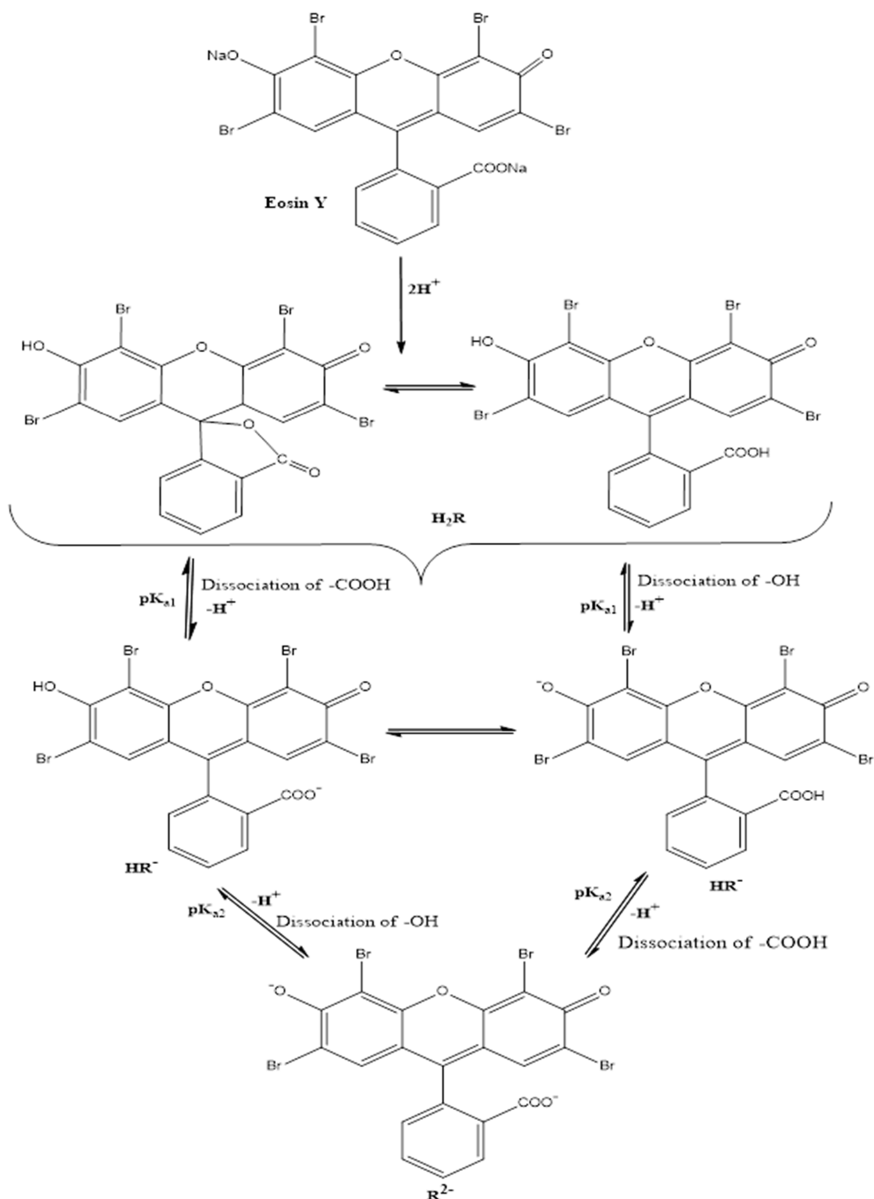

Fig. 3: Ionization of eosin Y and its three different forms (H_2R, HR^- and R^{2-}) [3-4]

In recent years, eosin was used as photoredox catalyst in organic synthesis [9], Carbon-Carbon and Carbon–Phosphorous bond formation [10]. It was utilized in the cleavage of C-C double bond of styrene [11], binding and estimation of protein assay [12-14], antimalarial agent for drug resistant *Plasmodium falciparum* [15], activating agent in teeth whitening composition [16], tracer in groundwater studies [17], complexing agent in ternary ion-association complex nanoparticles [18-19], sensing material in preparation of eosin Y film modified glassy carbon electrode [20], photosensitizer in dye sensitized solar cells and topical agents for treatment of diaper dermatitis and interfering agent in measurement of serum vancomycin [21-23]. Indirect determination of histamine, an important compound in various physiological processes in humans was determined in fish samples, dairy products

and alcoholic beverages complexation with Fe (III) and eosin Y [24]. Catalyst-free activation of peroxides through photoexcited electron under visible LED light irradiation was also performed using eosin Y as a model dye [25]. A highly selective and sensitive resonance light scattering detection approach was developed for the synchronous analysis of anthelmintics after reaction with eosin Y to form ion-association complexes and separated by high-performance liquid chromatography [26]. An environmentally useful method was developed to detect sodium dodecyl sulphate using eosin Y and polyethyleneimine complex [27].

Applicability of eosin Y in drug analysis

Several commercially available xanthene dyes have been successfully used in the analysis of many pharmaceutical compounds. Eosin Y is an acidic dye belongs to the xanthene group of dyes that has been widely used for the determination of several basic drugs through the formation of colored or fluorescent ion association complex using spectrophotometric and/or spectro-fluorimetric methods.

Formation of binary and ternary complexes

Binary ion association complexes formed between eosin and the drug molecules by electrostatic interaction. It was reported that the formation of binary complex increases the sensitivity of determination. It may decrease the fluorescence intensity of the native fluorescence of the eosin [28] without any permanent change in the molecule. The stability and fluorescence capacity of the ion association complexes were studied by using various acidic buffer solutions and dispersing agents. It was reported that pH is the critical factor which plays an important role on ionization of eosin Y. It was found that the fluorescence capacity of the ion association complex formed with eosin Y was increased up to certain pH values and then a decrease in the fluorescence intensity was occurred [29-31]. Dispersing agents also affect the stability of the complexes and prevents precipitation of the ion association complexes [32-33].

Ternary complex categorized into two major types, the one is mixed ligand complex and the other is an ion-association complex. Both types of ternary complex formation improve not only the sensitivity but also the selectivity as well. The sensitivity of the ternary complex can also enhance by the addition of surfactant that dissolves the ternary complex and measured directly without involving extraction process. In general, a ternary complex has formula $L_nM_xS_y$, where L stands for main ligand i.e. cited drug, M is the metal and S is the eosin Y, respectively.

The mechanism of ternary complex formation includes coordinate bond formation between metal ion and the cited drug molecule through atoms carrying lone pairs of electrons, and subsequently, a ternary complex is formed by interaction with eosin Y. The major advantage of ternary complex formation that it often has higher values of molar absorptivity and therefore improves the sensitivity of the method. Metal chelates formation also promotes the fluorescence and forms a ternary complex with eosin Y which can be utilized in the indirect determination of metals using atomic absorption spectrophotometric technique. These complexes are extractable with organic solvents such as chloroform and methylene chloride and have been widely utilized in the analysis of several pharmaceuticals. Eosin Y was used for determination of various pharmaceutical compounds using spectrophotometry and/or spectrofluorimetry through the formation of ternary complexes. However, the metal-drug and metal-eosin binary systems cannot be extracted in the same manner.

Quenching fluorescence

The term quenching refers to a process that decreases the fluorescence intensity of a given substance. It occurs during excited state lifetime and results in various types of molecular interactions. Collisional or dynamic quenching and static quenching are the two main types of quenching process. Both types of quenching require interaction between the fluorophore and quencher molecule. In dynamic quenching, the quencher molecule must diffuse to the fluorophore during excited state lifetime and upon contact, the fluorophore returns to the ground state without photon emission. Static quenching occurs when a fluorophore and quencher create a non-fluorescent complex before excitation of fluorophore [34]. Molecular oxygen, iodide, bromide ions and acrylamide are common quenchers for almost all dyes [35-38].

In addition to the above, apparent quenching can also occur due to the optical properties of the samples which are not much more useful. This trivial type of quenching occurs due to high optical densities or turbidity and can easily be controlled.

A separate type of quenching known as Fluorescence Resonance Energy Transfer (FRET) in which the intensity of the donor decreases and transfer to an acceptor molecule. The acceptor can be fluorescent or non-fluorescent. In both cases, the fluorescence intensity of the initially excited molecule is decreased. This type of quenching is often referred to as a donor-acceptor pair. It is mediated by the emission of a photon and it does not even require that the acceptor chromophore be fluorescent. During photosynthesis, a light antenna pigment uses resonance energy transfer to donate the collected energy to the photosynthetic reaction centre. This technique has led to qualitative and quantitative improvements, including increased spatial resolution and sensitivity. FRET is commonly used to measure distances within or between molecules in protein studies [39]. At present, FRET was used for measuring the structure [40-42], conformational changes [43] and interactions between molecules [44-45].

Applications of spectrofluorimetry in drug analysis using eosin

Spectrofluorimetry or fluorescence spectroscopy is a type of molecular emission spectroscopy and an extremely sensitive analytical method which has been widely applied for the determination of a variety of pharmaceutical compounds. It measures the fluorescence intensity which allows sensitive and selective quantitation of certain compounds which exhibit the fluorescence. This method involves measurement of enhanced or quenched fluorescent signals. It has been applied in three different processes: fluorescence, phosphorescence and chemiluminescence. Fluorescence is the most common luminescence process used in the pharmaceutical analysis. It involves photoexcitation process which occurs by absorption of various types of radiant energy such as UV-visible light and emission process in which emission radiant energy from an excited electronic state takes place as fluorescence within 10^{-6} to 10^{-9} seconds. Mostly fluorescent molecules are aromatic in nature. However, substituents such as-NH_2-OH,-F,-OCH_3,-$NHCH_3$ and $N(CH_3)_2$ groups, often enhances fluorescence while electron withdrawing groups containing halogen such as–Cl,-Br,-I, and-$NHCOCH_3$,-NO_2 or-COOH decrease or quench completely the fluorescence. Presence of dissolved oxygen, changes in buffer solutions of different pH values and solvent polarity also exhibit marked effect on the fluorescence of compounds.

This technique is important because of the fact that the intensity of light emitted by a fluorescent compound depends upon the concentration of that compound and hence, the measurement of fluorescence intensity permits the quantitative determination of trace contaminants of many inorganic species in the environment, industries and bodies. Fluorescence spectroscopy applied to drug analysis provides analytical methodologies with improved sensitivity, selectivity and range. It is one of the most important analytical methods in the field of chemistry, biology and chemical engineering. It is extensively used in nuclear research for the determination of uranium salts. It is also a method of the choice for the determination of many pharmaceutical compounds, plant pigments, hormones, food products, steroids and vitamins in formulations and biological fluids [46].

Taking the advantage of fluorescent properties of eosin Y, several researchers utilized eosin as a fluorescence quenching agent and analyses a variety of organic compounds. Eosin commonly used as an acidic red stain for highlighting cytoplasm material in samples. It forms either binary or ternary complex with pharmaceuticals and measured either directly without extraction or indirectly by extraction in an organic solvent. Many pharmaceutical compounds were analyzed using spectrofluorimetric technique involving eosin as a reactant. It was used in the analysis of various illicit drug

samples encountered in small amounts [47]. Coumarins were determined in a well-known traditional Indian drug '*Shankhpushpi*' [48], determination of scopoletin and mangiferin curcumin and testosterone in biological fluids [49-51].

The spectrofluorimetric method is less tedious and less cumbersome compared to chromatographic methods which require long run time and suffer from tedious operation procedures. Moreover, the high sensitivity and specificity offered by this method are also higher and the drug compounds can be analyzed up to nano levels. As data are shown in table 1, these methods were widely used in quantitation of many pharmaceuticals compounds using eosin Y due to its simplicity, low cost, high sensitivity and wide concentration range [52-68].

Applications of spectrophotometry in drug analysis using eosin

Spectrophotometry is a type of absorption spectroscopy. It involves measuring the amount of ultraviolet or visible light absorbed by a substance in solution. It is one of the most frequently used methods for the quantitation of pharmaceuticals due to its low cost, ease of operation and simplicity. It has been regarded as one of the most suitable and economical methods in research laboratories, hospitals and pharmaceutical industries. Visible region spectrophotometric methods found suitable for single component analysis [69, 70]. However, these methods were found not suitable for the multicomponent mixture. The instrumental development of ultraviolet region absorption spectrophotometry was reviewed and applied to solve multicomponent pharmaceutical mixture [71]. Eosin Y formed a binary or ternary colored nonfluorescent complexes were analyzed by spectrophotometry. A number of pharmaceutical compounds were analyzed using eosin Y as an ion pairing agent shown in table 2 [72-93].

Comparison of spectrofluorimetric and spectrophotometric methods of analysis using eosin y

It has been reported that eosin Y is a common dye used for determination of various pharmaceutical compounds either spectrophotometry alone or spectrophotometry and spectrofluorimetry by the formation of a binary or ternary complex. Fig. 4 shows a schematic explanation that how the ion association complex formed with eosin Y and analyzed by spectrophotometry and spectrofluorimetry.

Table 1: Spectrofluorimetric determination of drugs using eosin Y

Drug	Complex type	λ_{em} (nm)	λ_{ex} (nm)	Range µg/ml	LOD[a] µg/ml	LOQ[b] µg/ml	Application	Reference
Olanzapine	Ternary	547	323	0.05-1.0	0.0018	0.006	Pharmaceutical preparations and human plasma	52
Fluphenazine	Ternary	547	323	0.10-1.0	0.0012	0.004	Pharmaceutical preparations and human plasma	52
Risperidone	Ternary	555	260	0.5-7	0.015	0.050	Bulk and tablets	53
Labetalol	Ternary	452	317	0.5-4	0.08	0.23	Pharmaceutical preparations and urine	56
Doxepin HCl	Binary	567	464	0.1-8	0.00295	0.00975	Commercial dosage forms	54
Betaxolol	Binary	545	301.5	0.1-2.5	0.028	0.086	dosage forms	55
Carvedilol	Binary	545	301.5	0.1-2.5	0.024	0.071	dosage forms	55
Labetalol	Binary	545	301.5	0.1-2.5	0.057	0.172	dosage forms	55
Nebivolol	Binary	545	301.5	0.1-2.5	0.046	0.14	dosage forms	55
Propranolol	Binary	545	301.5	0.1-2.5	0.016	0.05	dosage forms	55
Labetalol	Binary	432	312	1.25-30	0.24	0.73	Pharmaceutical preparations and urine	56
Chloroquine	Binary	372	318	0.5–5	-	-	Dosage forms	57
Amodiaquine	Binary	368	318	0.5-8	-	-	Dosage forms	57
Primaquine	Binary	450	368	0.1–5	-	-	Dosage forms	57
Clindamycin HCl	Binary	555	482	0.2-2	0.13	0.18	Pure and dosage forms	58
Almotriptan malate	Binary	542.8	301.3	0.07-1.0	0.019	0.059	Pure and dosage forms	59
Rizatriptan benzoate	Binary	542.8	301.3	0.20-1.0	0.041	0.125	Pure and dosage forms	59
Sumatriptan succinate	Binary	542.8	301.3	0.20-1.0	0.055	0.168	Pure and dosage forms	59
Zolmitriptan	Binary	542.8	301.3	0.10-1.0	0.032	0.096	Pure and dosage forms	59
Losartan	Binary	546	310	0.8-8	0.203	0.617	Tablets	60
Irbesartan	Binary	546	310	0.8-7	0.110	0.335	Tablets	60
Telmisartan	Binary	546	310	0.9-4	0.112	0.340	Tablets	60
Valsartan	Binary	546	310	1-8	0.132	0.399	Tablets	60
Amitriptyline HCl	Binary	550	310	0.08-2	0.017	0.056	Pharmaceutical preparations	61
Clomipramine HCl	Binary	550	310	0.06-2	0.015	0.049	Pharmaceutical preparations	61
Citalopram HBr	Binary	554	259	2-26	0.121	-	Dosage forms	62
Fluoxetine HCl	Binary	545	301	0.2-2.4	0.066	0.036	Pharmaceutical formulations	63
Paroxetine HCl	Binary	545	301	0.1-2.4	0.20	0.10	Pharmaceutical formulations	63
Ethionamide	Binary	536	337	1-8	0.08	-	Pharmaceutical preparations and biological fluids	64
Thioridazine	Ternary	517	462	0.5-3	-	-	Dosage forms	65
Flupentixol	Ternary	517	462	0.5-3	-	-	Dosage forms	65
Sunitinib malate	Binary	800	350	0.08-5	0.041	0.85	Bulk and pharmaceutical preparations	66
Ebastine	Binary	553	457	0.1-1.0	0.021	0.042	Pharmaceutical preparations	67
Prochlorperazinedimaleate	Binary	450	318	1-10	-	-	Pharmaceutical preparations	68
Thiethylperazinedihydrochloride	Binary	460	318	1-10	-	-	Pharmaceutical preparations	68
Trifloperazinedihydrochloride	Binary	465	318	1-10	-	-	Pharmaceutical preparations	68

[a]Limit of detection, [b]Limit of quantitation.

Table 2: Spectrophotometric determination of drugs using eosin Y

Drug	Complex type	λ_{max} (nm)	Range µg/ml	LOD µg/ml	LOQ µg/ml	Application	Reference
Tizanidine	Binary	545	0.5-8	0.1	0.26	Dosage forms	72
Orphenadrine	Binary	542	1-12	0.3	0.95	Dosage forms	72
Clemastine hydrogen fumarate	Binary	552	1.25-11.25	0.72	2.39	Dosage forms	73
Desloratadine	Binary	549	0.31-2.81	0.9	3	Dosage forms	73
Losartan potassium	Binary	540	2.5-20	0.82	2.73	Dosage forms	73
Moxepril HCl	Binary	540	1.25-15	0.75	2.51	Dosage forms	73
Bezafibrate	Ternary	546	0.06-3	0.00915	0.0277	Pharmaceutical products	74
Amlodipine	Binary	549	5-60	1.8	6.0	Bulk powder and pharmaceutical formulations	74
Nicardipine	Binary	549	5-60	1.2	3.6	Bulk powder and pharmaceutical formulations	74
Terbutaline sulphate	Binary	545	0.5-10	0.030	0.103	Pharmaceutical formulations	75
Tetracycline hydrochloride	Binary	545	5-45	0.613	2.00	Pharmaceutical formulations	75
Erythromycin	Binary	542-544	2-20	0.172	0.565	Pharmaceutical formulations and biological fluids	76
Azithromycin	Binary	542-544	1-10	0.153	0.514	Pharmaceutical formulations and biological fluids	76
Clarithromycin	Binary	542-544	3-30	0.281	0.906	Pharmaceutical formulations and biological fluids	76
Roxithromycin	binary	542-544	2-20	0.253	0.849	Pharmaceutical formulations and biological fluids	76
Ramipril	Ternary	535	20-100	-	-	Tablets	77
Perindopril	Ternary	535	10-60	-	-	Tablets	77
Enalapril	Ternary without surfactant	533.4	56-112	1.412	-	Dosage forms	78
Enalapril	Ternary with surfactant	558.8	20-32	0.587	-	Dosage forms	78
Gatifloxacin	Ternary	552	2-10	0.216	0.72	Pharmaceutical formulations	79
Moxifloxacin	Ternary	549	1-8	0.184	0.613	Pharmaceutical formulations	79
Memantine HCl	Binary	546	1-10	0.33	0.99	Tablets	80
Perindopril erbumine	Ternary	510	10-200	0.49	1.48	Pharmaceutical preparations	81
Gliclazide	Ternary	550	0.5-4	0.05	0.5	Pharmaceutical formulations and biological fluids	82
Levofloxacin	Binary	547	2-8	0.1475	-	Pure, pharmaceutical tablets and spiked human urine	83
Norfloxacin	Binary	547	2-8	0.1402	-	Pure, pharmaceutical tablets and spiked human urine	83
Ciprofloxacin	Binary	547	2-8	0.1369	-	Pure, pharmaceutical tablets and spiked human urine	83
Olanzapine	Ternary	540	0-35	0.1501	0.4547	Pure and Dosage Forms	84
Orphenadrine	Ternary	540	0-55	0.3109	0.9422	Pure and Dosage Forms	84
Cefixime	Ternary	550	4-28	0.90	2.70	Dosage Forms	85
Glimepiride	Ternary	544	5-50	1.70	5.10	Dosage Forms	86
Sparfloxacin	Ternary	550	1.6-16	0.0211	0.0704	Bulk and pharmaceutical preparations	86
Minocycline	Ternary	545	0-4	-	-	Pharmaceutical preparations	87
Meclizine	Binary	540	5-25	0.76	2.29	Pure and dosage forms	88
Tolterodine tartrate	Binary	545	1-10	0.10	0.31	Pharmaceutical preparations	89
Berberine sulphate	Binary	-	-	-	-	Tablets	90
Solifenacin succinate	Ternary	545	2.5-50	0.116	0.351	Dosage forms	91
Metoclopramide HCl	Binary	543	1.01-10.09	0.124	0.414	Dosage forms	92
Carbinoxamine	Ternary	538	0.75-10	-	-	Pharmaceutical formulations	93

It was found that the binary or ternary complex formed by interaction with eosin alone or eosin and metal measured through spectrofluorimetry offers high sensitivity and selectivity compared to the spectrophotometric analysis. A list of pharmaceuticals reported in table 3 clearly states that spectrofluorimetric methods are more sensitive and have low limit of detection [94-101].

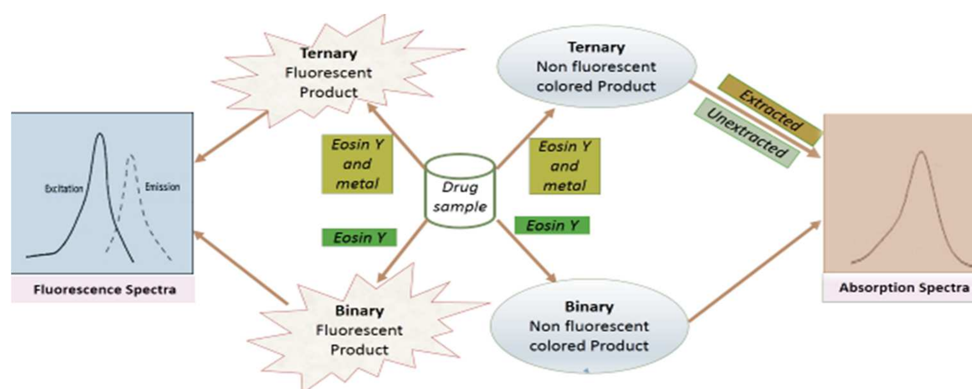

Fig. 4: Schematic diagram for ion association complexation with eosin Y and its analysis

Table 3: Comparison of spectrophotometric and spectrofluorimetric determination of drugs using eosin Y

Drug	Complex type	Spectrophotometry				Spectrofluorimetry					Application	Reference
		λ_{max} (nm)	Range µg/ml	LOD µg/ml	LOQ µg/ml	λ_{em} (nm)	λ_{ex} (nm)	Range µg/ml	LOD µg/ml	LOQ µg/ml		
Dothiepin HCl	Binary	540	1-10	0.18	0.54	543	304	0.3-8	0.11	0.34	Pure and dosage forms	94
Hydrochlorothiazide	Ternary	543	8-40	0.046	0.138	545	462	0.05-0.25	0.013	0.039	Tablets	95
Indapamide	Ternary	543	8-40	0.041	0.123	545	462	0.05-0.25	0.014	0.044	Tablets	95
Xipamaide	Ternary	543	8-32	0.035	0.107	545	462	0.05-0.25	0.012	0.039	Tablets	95
Ciprofloxacin	Ternary	545	3-10	0.142	0.431	540	310	0.0375-0.070	0.0018	0.0055	Pure and tablets	96
Norfloxacin	Ternary	545	3-10	0.138	0.419	540	310	0.025-0.050	0.0010	0.0033	Pure and tablets	96
Ropirnirole	Binary	546	50-500	-	-	540	350	6-150	-	-	Dosage forms	97
Mebeverine HCl	Binary	551	1-12	0.53	1.04	540	390	0.2-3.5	0.11	0.21	Commercial Tablets	98
Doxazosin mesylate	Binary	547	2-14	0.393	1.191	570	430	1-10	0.0794	0.241	Tablets	99
Astemizole	Ternary	547.5	4.1-37.6	-	-	545	462	0.94-7.1	-	-	Tablets, Suspension and capsule	100
Terfenadine	Ternary	540.7	11.8-47.2	-	-	545	462	0.94-7.1	-	-	Tablets, Suspension and capsule	100
Flunarizine HCl	Ternary	547.5	2.4-19.1	-	-	545	462	0.94-7.1	-	-	Tablets, Suspension and capsule	100
Clopidogrel	Binary	545	0.5-9	0.076	0.23	560	499	0.2-6	0.0341	0.1033	Pharmaceutical preparation	101

CONCLUSION

As revealed above eosin Y is one of the important fluorescent ion pairing agent and spectrofluorimetric technique play an important role in the analysis of many pharmaceutical compounds and active current research area as a number of research articles and reviews published every year on this topic [102-108]. Spectrofluorimetry provides an extremely sensitive, selective and simple method for a variety of active pharmaceutical ingredients at a very low detection limits up to pictogram range [109]. A number of pharmaceutical compounds were also analyzed by spectrophotometry that formed a binary and/or ternary colored complex with eosin Y which proves that it is also an important technique of choice even today in pharmaceutical industries. However, the binary and ternary complex formed with eosin Y and analyzed by spectrofluorimetry offered higher sensitivity and selectivity over spectrophotometry.

Chromatographic methods such as high-performance liquid chromatography and gas chromatography were used as a valuable tool for the quantitative analysis of several pharmaceutical ingredients. However, non-chromatographic methods such as spectrofluorimetry and spectrophotometry are still extensively used in research laboratories, hospitals due to low cost, simplicity, portability and ease of operation. Both nonchromatographic methods involved in analyses utilizing the quenching nature of various fluorescent dyes and hence can be used as an alternative to chromatographic methods using eosin Y as an ion pairing agents for a variety of pharmaceutical compounds. These two methods can be recommended for routine quality control analysis of drugs where time, cost effectiveness and high specificity of analytical techniques are of great importance.

CONFLICT OF INTERESTS

Declared none

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