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SPECTROPHOTOMETRIC DETERMINATION OF DRUGS AND PHARMACEUTICALS BY USING FOLIN-CIOCALTEU REAGENT

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ABSTARCT

A simple, fast, selective and accurate spectrophotometric method has been outlined for the assessment of five phenolic drugs namely Balsalazide, Desvenlafaxine, Formoterol, Nelfinavir mesylate, Xipamide. This method is based on the development of blue coloured chromogen due to reduction of tungstate and/ or molybdate in Folin-Ciocalteu reagent by Drug in alkaline medium. The coloured species has an absorption maximum at 770nm, 755nm, 735nm,760nm, 740nm and the Beer's law obeys over the concentration range of 7–48µg/mL, 2-14 µg/mL, 5-35 µg/mL, 3.5-24.5 µg/mL, 11-77 µg/mL respectively for the drugs Balsalazide, Desvenlafaxine, Formoterol, Nelfinavir mesylate, Xipamide. This method has been validated according to ICH guidelines and applied to the analysis of pharmaceuticals**.**

KEYWORDS: Spectrophotometry, Folin-Ciocalteu reagent, drugs, pharmaceutical formulations.

1. INTRODUCTION

1. Balsalazide [BSZ] is chemically known as (E)-5-[[-4-[[(2 carboxyethyl)amino]carboxyl] pheny]azo]-2-hydroxy benzoic acid, disodium dihydrate. It is a prodrug that is enzymatically divided in the colon to deliver mesalamine (5-aminosalicylic acid), an anti-inflammatory drug (NSAID) which is useful for treatment of active ulcerative colons and gastrointestinal disorders such as inflammatory bowel disease. Balsalazide and one of its primary metabolites prevent the growth of cultivated human colon cancer cells and Balsalazide inhibit abnormal crypt formation in carcinogenic azoxymethane-treated animals. It is utilized in the treatment of mild to moderate ulcerative colitis.[1-3] Balsalazide disodium capsules comprise Balsalazide

disodium granule that are insoluble in acid and are meant to be administered to the colon intact. Once bacterial azo reductases enter the colon, break the compound to release 5-aminosalicylic acid, the molecule's therapeutically active component, and 4-aminobenzoyl-β-alanine.[4] Literature survey found many analytical methods for the determination of Balsalazide. These methods include UV Spectrophotometric method[4-6], HPLC[7-8] and Liquid Chromatography.[9]

2. Desvenlafaxine [DVL] is chemically designated as RS-4-[2-dimethylamino-1-(1 hydroxy cyclohexyl) ethyl]phenol. It isa serotonin-nor epinephrine reuptake inhibitor class antidepressant, is used in the treatment of depression. Desvenlafaxine is a synthetic form of the isolated major active metabolite of venlafaxine and is grouped as a selective serotonin and norepinephrine reuptake inhibitor.[10] Literature survey reveals that methods have been reported for analysis of desvenlafaxine by UV-Visible spectroscopy[11-13], HPTLC[14-15], Liquid Chromatography[16], UPLC-TMS[17], LC-UV & LC-MS[18], Spectrofluorimetric method[19], Voltammetry.[20]

3. Formoterol [FMT] chemically known as N-[2-hydroxy-5-[1-hydroxy-2-[1-(4-methoxy phenyl) propan-2-ylamino]ethyl]phenyl]formamide. Formoterol is a long-acting β-2 agonist used for asthma and chronic pulmonary obstructive disease. It is a bronchodilator b- receptor with impressive Broncho selectivity, anti-allergic activity and pulmonary edema- inhibitory effect.[21-22] A survey of the literature revealed that a few analytical methods have been reported for the determination of formoterol by UV-Visible Spectrometry[22-23], HPLC[24-27], HPTLC[28], Capillary Electrophoresis[29], Gas Chromatography[30], NMR spectroscopy.[31]

4. Nelfinavir mesylate (NFM) is a novel HIV-1 protease inhibitor. Its chemical name is (3S,4aS,8aS)-N-tert-butyl-2-[(2R,3R)-2-hydroxy-3-[(3-hydroxy-2methylphenyl) formamido]-4-(phenylsulfanyl)butyl]-decahydroisoquinoline-3-carboxamide methane sulfonate. It is the mesylate salt of a simple amine and has shown an effective and selective protease inhibitor for human immunodeficiency virus 1 (HIV-1). Nelfinavir mesylate has been shown to substantially reduce viral load and increase CD4+ T cells in adults and children infected with HIV, especially when administered in combination with other anti-HIV agents, nucleoside analogues, reverse transcriptase inhibitors and protease inhibitors.[32]. NFM has been determined by many analytical methods, such as; Spectrophotometric method[33], HPLC method[34-37], HPTLC method[38], LC-MS[39-40], Liquid Chromatography[41], RPLC.[42]

5. Xipamide (XPM) is chemically 4-chloro-N-(2,6-dimethylphenyl)-2-hydroxy-5-sulfamoylbenzamide. It is a non-thiazide diuretic with a more natriuretic effect than the thiazides and a less rapid onset and longer duration of action than furosemide. It has a moderately strong diuretic action. It lowers active sodium re-absorption and subsequent chloride by binding and inhibiting its action to the chloride site of the electro neutral Na+/Cl- co-transport system. Xipamide provides an effective alternative to other diuretics in the treatment of patients with mild to moderate hypertension and oedema patients for a variety of causes.[43] The literature survey reveals several analytical methods for the determination of Xipamide including Spectrophotometric.[44-47], Spectrofluorimetric[46-48], HPLC.[49-51], HPLC-DAD[52], Voltammetry.[53]

Chromatographic methods are the most widely used ones. Although various techniques listed above are specific, most of the described techniques are time consuming and require multistage extraction procedures. On the other hand, the mentioned spectrophotometric techniques are for numerous reasons not satisfactory for the routine quality assurance. Some of these techniques suffer from drawbacks such as poor sensitivity, low species stability, use of organic solvent, scrupulous monitoring of experimental variables and special equipment.

Folin-Ciocalteu (F-C) reagent is commonly used in plant biology to evaluate polyphenols and to assess other phenolic compounds in pharmaceuticals based on a reduction of Folin-Ciocalteu reagents. This paper outlines a reduction process involving F-C reagent and drugs leading to the formation of a blue coloured chromogen that can be tested at 740nm- 770nm range for respective drugs.

STRUCTURE OF DRUGS

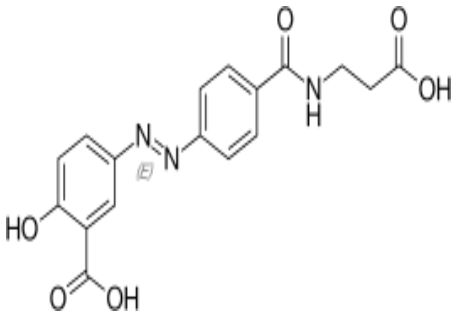

Fig 1: Balsalazide

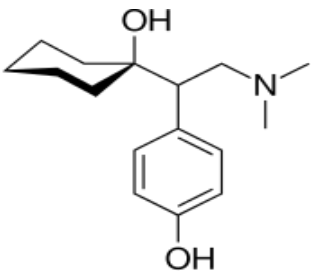

Fig 2: Desvenlafaxine

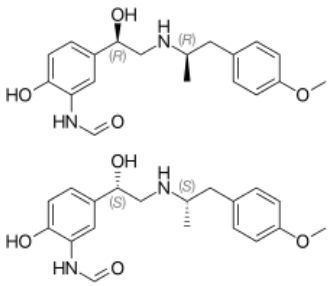

Fig 3: Formoterol

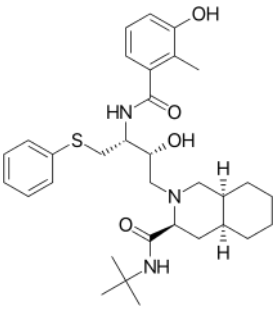

Fig 4: Nelfinavir mesylate

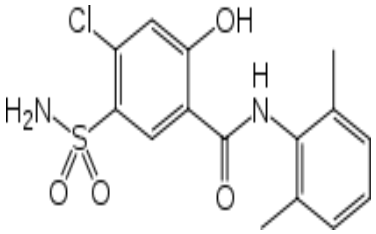

Fig 5: Xipamide

2. MATERIALS AND METHODS

2.1 Instrumentation

All absorption spectra were recorded on SHIMADZU 2600 UV-Visible double beam spectrophotometers using quartz cells of 10mm path length. A Dhona 200 single pan electrical balance is used for weighing the samples.

2.2 Materials and Reagents

All chemicals and reagents used were of analytical or pharmaceutical grade. Double distilled water was used throughout the investigation. Folin-Ciocalteu reagent (Merck, Mumbai), Sodium carbonate (Hetero Drugs Pvt Ltd, Hyd) were of analytical reagent grade and used without further purification. The pharmaceutical grade pure drug samples were kindly provided by GVK biosciences Pvt Ltd, Hetero Drugs Pvt Ltd, Dr Reddy's Laboratories, Hyderabad.

A stock standard solution of each drug containing 100 µg/mL were prepared by dissolving accurately weighed 10 mg of the respective drug transferred in to a 100 mL volumetric flask and made up to mark with distilled water. The solutions were further diluted quantitatively according to their linearity range. The pharmaceutical preparations were purchased from a local market and analysed.

2.3 Method development

Different aliquots of the drug solution (1 to 7 mL) were transferred into a series of 10 mL calibrated flasks. To each flask, 1 mL 10% Na_2CO_3 was added, followed by 2 mL 2N Folin-Ciocalteu reagent. The contents were mixed and they were set aside for 20 minutes under occasional shaking. The volume was made up to the mark with water and the absorbance of each solution was measured at 740-770nm range against a reagent blank.

2.4 Construction of calibration curves

The calibration curve was plotted by taking concentration of the drugs in X-axis and absorbance in Y-axis. The calibration curves were constructed by taking absorbance data in six replicate experiments. The absorbance to concentration called relative response is calculated. Those points falling between 95% to 105% of the average relative response are only considered for construction of calibration.

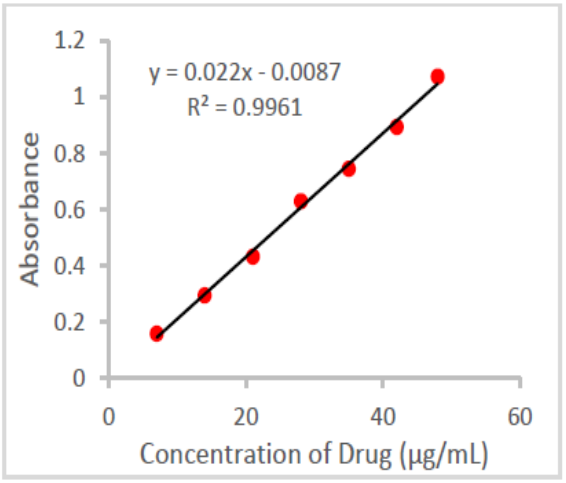

Fig 6: Calibration curve of Balsalazide

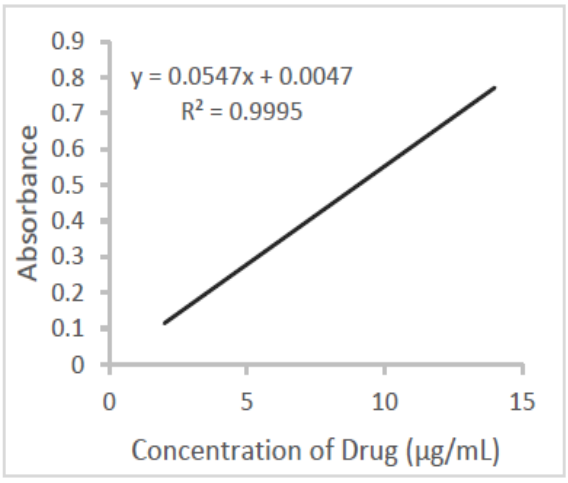

Fig 7: Calibration curve of Desvenlafaxine

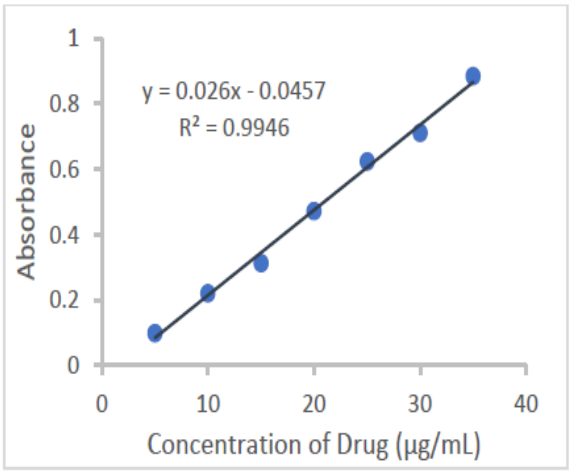

Fig 8: Calibration curve of Formoterol

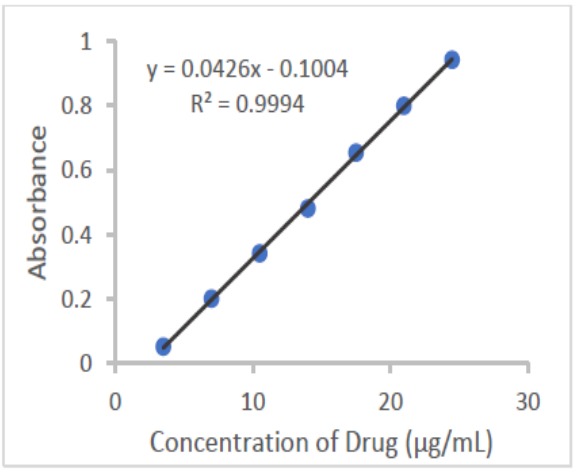

Fig 9: Calibration curve of Nelfinavir mesylate

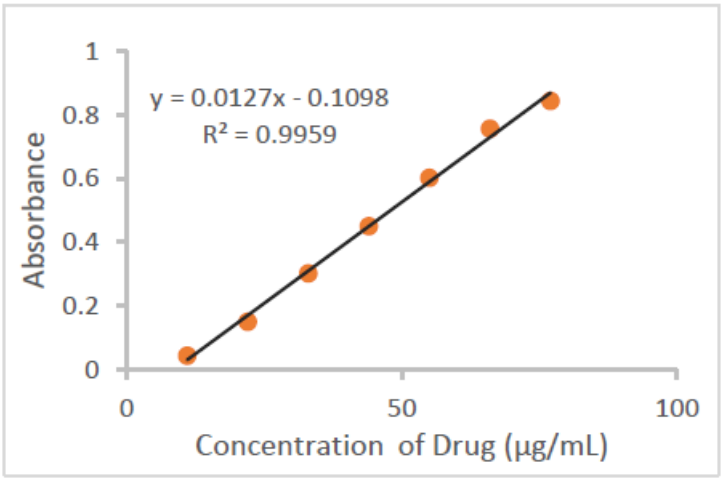

Fig 10: Calibration curve of Xipamide

2.5 Accuracy and Precision studies

Accuracy of the methods developed are evaluated from the recovery studies on pure drug sample. At least four known concentration of drug solutions have been brought into Beer's law limit and recovery studies have been carried out. Excellent recovery showed the validity of the calibration curves for each drug. Precision of the procedure is illustrated by repeating experiment (n=6) and %RSD is found out. %RSD being less than 1 in each case speaks the high precision of the method.

2.6 Procedure for analysis of pharmaceuticals Balsalazide

Two tablets (BALACOL, 750mg) were weighed and crushed in to fine powder. Powder weight equivalent to 10mg of Balsalazide was transferred into 100mL calibrated flask and made up to mark with water. And the solution filtered through a Whatman No. 42 filter paper. It was used as stock sample solution and was further diluted with distilled water to get working concentration solution for analysis of the drug.

Desvenlafaxine

An amount of finely ground tablet powder (D-VENIZ, 50mg) equivalent to 10 mg of Desvenlafaxine was accurately weighed in100 mL volumetric flask, the flask was shaken after addition of a 50 mL of water for about 5 minutes and the volume was made up to the mark with distilled water. The solution was kept aside for 20 minutes and filtered using Whatman No. 42 filter paper. The first 10 mL portion of the filtrate was thrown away and an appropriate aliquot as described under "General Analytical Procedure" was used for the assay.

Formoterol

For the determination of pharmaceutical formulations about 10 to 15 tablets (DERIFORM, 12mg) were weighed and powdered. A weight equivalent to 10mg of Formoterol was transferred into a 100 mL volumetric flask and finally volume was diluted up to the mark with distilled water, mixed well and the solution was kept aside for 20 minutes. After 20 minutes solution filtered using a Whatman No. 42 filter paper. First 10ml portion of the filtrate was discarded and a suitable aliquot of the subsequent portion was diluted appropriately to get working concentration and the assay was completed according to the procedure described above.

Nelfinavir

Three tablets (NEL, 250mg) were weighed and ground in to fine powder. A quantity equivalent to 10mg of Nelfinavir has been taken into a 100 mL of calibrated flask and added to about 30mL of methanol, sonicated for 30 minutes and filtered through Whatman filter paper No-42. The residue was washed thrice with methanol for complete recovery of drug and methanol was evaporated. The residue was dissolved in 100mL of distilled water. It was used as a stock sample solution. Aliquot portions of this stock solution were further diluted with distilled water to get the final concentration required for the determination of the drug.

Xipamide

About 15 to 20 tablets (XIPAMID, 20mg) were accurately weighed and crushed to a fine powder and the powder equivalent to 10mg of Xipamide was weighed accurately and transferred to 100mL volumetric flask, 60 mL of water was poured into the flask after that flask was shaken for about 5 minutes and the volume was made up to the mark with distilled water. The solution was kept aside for 20 minutes and filtered using Whatman No. 42 filter paper. First 10ml portion of the filtrate was discarded and an appropriate aliquot of the subsequent portion was diluted appropriately to get working concentration and the determination was performed according to the procedure described above.

The drug solutions extracted from tablet formulations were subjected to redox reaction with Folin-Ciocalteu reagent and subsequent determination was done. The concentration of tablet solutions falling under Beer's law limit was chosen for drug assay in the tablet. An outstanding tally between the concentration of drugs taken and found indicated the applicability of methods for formulations.

3. RESULTS AND DISCUSSIONS

The F-C reagent is used to evaluate several phenolic compounds and a great number of pharmaceutical-interest substances. The structural features of mentioned drugs permitted Folin-Ciocalteu reagent to be used for its analysis. The proposed technique is based on the development of a blue coloured chromogen, following the reduction of phosphor-molybdo tungstic mixed acid of the F-C reagent by phenolic drugs, in the presence of sodium carbonate, which could be estimated at 740-770nm range for respective drugs. The mixed acids in the F-C reagent are the final chromogen and involve the following chemical species.
3H2O.P2O5 .13 WO3 .5MoO3 .10H2O and 3H2O.P2O5 .14 WO3 .4 MoO3 .10H2O.

The F-C reagent is assumed to contain heteropolyphospho-tunstatesmolybdates. Drugs are likely to reduce tungstate and/or molybdate oxygen atoms in the F-C reagent by producing one or more possible reduced species which have characteristic intense blue colour.

3.1. Analytical data

Under optimum conditions a linear correlation was found between absorbance at λ max and concentration of all drugs in the ranges given in table 1. Sensitivity parameters such as molar absorptivity, Sandell sensitivity are also presented in Table 1. Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a), correlation coefficient (r) and is also given in table 1.

The LOD and LOQ were determined based on the standard deviation of the y-intercept and the slope of the calibration curves and presented in table1.
LOD = 3.3 a /b LOQ = 10 a/b.
Where a = standard deviation of the intercept (n = 6) b = slope of Calibration plot.

Table 1: Analytical Parameters for determination of drugs By Red-Ox reaction with Folin-Ciocalteu reagent.

Parameters	BSZ	DVL	FMT	NFN	XPM
λmax (nm)	770	755	735	760	740
Beer's law Limits (µg/mL)	7-48	2-14	5-35	3.5-24.5	11-77
Sandell sensitivity (µg cm^{-2})	0.0454	0.018	0.038	0.0234	0.0787
Limit of Detection (µg mL^{-1})	0.375	0.855	0.495	3.81	9.22
Limit of Quantification (µg mL^{-1})	1.136	2.59	1.5	11.57	27.95

Regression equation, Y	=0.022x-0.0087	=0.0547x+0.0047	=0.026x-0.0457	=0.0426x-0.1004	=0.0127x-0.1098
Intercept (a)	0.0087	0.0047	0.0457	0.1004	0.1098
Slope (b)	0.022	0.0547	0.026	0.0426	0.0127
Correlation coefficient (r)	0.998	0.999	0.997	0.999	0.997
SD of Intercept (Sa)	0.0025	0.014	0.0039	0.0493	0.0355
SD of Slope (Sb)	0.0047	0.0064	0.0045	0.0016	0.034
Molar Absorptivity ($L\ mol^{-1}\ cm^{-1}$)	8.16×10^3	1.55×10^4	6.87×10^3	8.65×10^3	1.3×10^3

Table 2: Determination of accuracy and precision of the methods on pure drug samples.

Drug	Taken (µg/mL)	Found (µg/mL)	Error (%)	Recovery (%)	RSD (%)	Proposed method mean±SD
BSZ	14.0 21.0 28.0 35.0	14.02 20.97 28.01 35.08	0.14 0.14 0.03 0.22	100.14 99.86 100.03 100.22	0.154	100.06±0.155
DVL	6.0 8.0 10.0 12.0	6.02 7.96 10.06 11.99	0.33 0.5 0.6 0.08	100.33 99.5 100.6 99.92	0.48	100.08±0.481
FMT	10.0 15.0 20.0 25.0	9.97 15.05 20.0 24.98	0.3 0.33 0.00 0.08	99.7 100.33 100.00 99.92	0.261	99.98±0.261
NFN	7.0 10.5 14.0 17.5	6.99 10.48 14.02 17.43	0.14 0.19 0.14 0.4	99.86 99.81 100.14 99.60	0.222	99.85±0.222
XPM	22.0 33.0 44.0 55.0	21.98 33.01 44.01 55.06	0.09 0.03 0.02 0.11	99.91 100.03 100.02 100.11	0.081	100.01±0.082

Table 3: Results of assay of tablets by proposed method and statistical evaluation and recovery experiments by standard addition method.

Drug	Taken (µg/mL)	Found (µg/mL)	Error (%)	Recovery (%)	RSD (%)	Proposed method mean± SD	Reference method mean±SD	t-Test	F-Test
BSZ	14.0 24.0 35.0 48.0	13.98 23.96 35.03 48.02	0.14 0.16 0.08 0.04	99.86 99.84 100.08 100.04	0.122	99.95±0.122	99.09±0.106	0.25	1.32
DVL	4.0 8.0 12.0	4.00 7.98 12.03	0.0 0.25 0.25	100.00 99.75 100.25	0.212	99.97±0.212	99.26±0.277	0.413	0.592

	16.0	15.98	0.12	99.88					
FMT	12.0 18.0 24.0 30.0	12.00 18.04 23.98 29.98	0.00 0.22 0.08 0.06	100.00 100.22 99.92 99.94	0.136	100.02±0.137	100.62±0.587	1.31	0.052
NFN	5.0 10.0 14.0 20.0	5.00 10.00 13.99 19.99	0.0 0.0 0.07 0.05	100.00 100.00 99.93 99.95	0.035	99.97±0.0355	100.3±0.057	0.328	0.3
XPM	18.0 28.0 35.0 45.0	18.04 27.98 35.03 44.96	0.22 0.11 0.08 0.08	100.22 99.89 100.08 99.92	0.152	100.02±0.153	100.6±0.53	0.685	0.082

4. CONCLUSION

A simple, selective, rapid and sensitive method has been proposed for the assay of drugs and in pharmaceutical formulations. The method is focused on the well-characterized and proven red-ox reaction and uses very simple, cheaper chemicals and easily accessible instruments. Other features like short performance time, ease of handling and the non-use of organic solvents also suggest this procedure as a routine laboratory method. The method is successfully applied to quantifying drugs in tablets and injecting them without intervention from common excipients. The current method is ideal for evaluation of BSZ, DVL, FMT, NFN and XPM in bulk drugs and pharmaceuticals. therefore, this method can be used in laboratories for quality control.

5. ACKNOWLEDGMENT

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