Journal of Pharmaceutical Research International

33(47B): 200-209, 2021; Article no.JPRI.76295
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Visible Quantitative Methods for the Estimation of Furosemide in Pure form and Pharmaceutical Formulations

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i47B33113
Editor(s):
(1) Mohamed Fawzy Ramadan Hassanien, Zagazig University, Egypt.
Reviewers:
(1) Naziya I. Khan, Appasaheb Birnale College of Pharmacy, India.
(2) Rakh Maharudra Shamrao, PVP College of Pharmacy, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/76295

Original Research Article

Received 13 August 2021
Accepted 26 October 2021
Published 01 November 2021

ABSTRACT

Aims: Design of technical methods for the determination of Furosemide in its pure and pharmaceutical dosage form using spectral methods.
Study Design: planned and executed to estimate Furosemide by using Visible spectrophotometric in pure and pharmaceutical dosage form.
Place and Duration of Study: Laboratory of Analytical Research, chemistry department, college of Science, University of Mosul ,Mosul-Iraq, during the period of April 2021 to August 2021.
Methodology: Furosemide, the commercially known drug Lazix, which is important in the treatment of heart diseases and high blood pressure. This study was carried out using JASCO V – 630, double-beam computerized UV-Visible spectrophotometer, with 1 cm matched cell, and HANA pH meter was used for reported pH readings.
Results: The reaction between Furosemide and bromo-phenol blue, xylenol orange, and chromazorol S. The decreasing in the intensity of the resulted colored complex was measured using bromo-phenol blue, xylenol orange, While the increasing of the color intensity was measured in the method (C). These three methods were based on charge transfer reaction. The limits of Beer's law for method (A) 0.4-32µg. mL^{-1}, method (B) 1-32 and method (C) were 0.8-32 depending on the level of concentration, while the values of the molar absorption coefficient 1.4×10^4, 2.1×10^4 and 1.57×10^4 $l.mol^{-1}.cm^{-1}$ for the first, second and third method respectively. Sandel's significance

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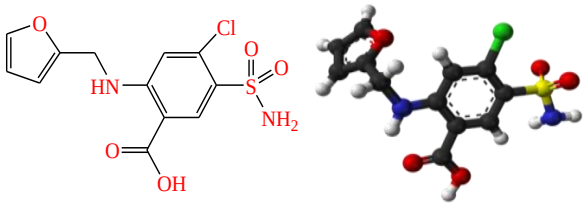
also was calculated for these three methods, 0.0157 µg.cm^{-2} for the first method, 0.0236 µg.cm^{-2} for the second method, while the third method was 0.0210 µg.cm^{-2}. The method has been successfully applied for the determination of furosemide in its pure form and in some of its pharmaceutical preparations

Conclusion: The proposed methods were validated in terms of linearity, range, Accuracy, precision, Specificity, Robustness. The proposed methods were successfully applied to the estimation of Furosemide in pharmaceutical dosage form, method (B) was experimentally considered as a best method depending on the best values of molar absorptivity, stability of the resulted complex, and the linearity of the method (B) .

Keywords: Furosemide; xylenol orange; bromo-phenol blue; chromazorol s; pharmaceutical preparations.

1. INTRODUCTION

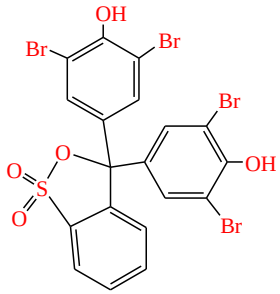
Furosemide (FSD) is known commercially as Lasix, and chemically as 4-chloro-en-furfuryl-5-sulfamoylantranilic acid or 5-(aminesulfonyl)-4-chloro-2[(2-furanylmethyl) amino] benzoic acid. FSD is used to treat high blood pressure as well as a diuretic where it is used to treat edema associated with heart failure, cirrhosis, and kidney disease [1-5].



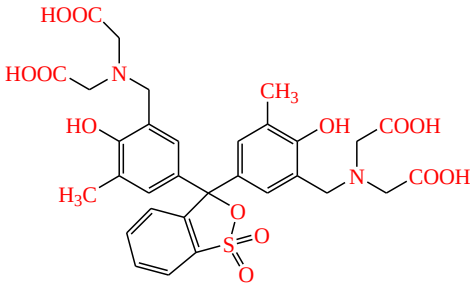
Chemical structure of Furosemide

FSD is an important drug for human health, as it is used to treat the most important organs of the body, starting with the heart, kidneys and liver. Therefore, researchers have dealt with this drug in many studies, which have dealt with its solubility, uses and methods of estimation it, mostly of these method used various techniques, and among of these techniques, a spectrophotometric and chromatographic methods have been used for determination of FSD depending on: charge transfer complex method [6], mass spectroscopy [7], infra-red spectroscopy [8], Also, first order derivative spectroscopy method and absorbance ratio (Q-Absorbance) method were used [9], other spectrophotometric method was using principal component regression [10], Validated RP-HPLC Method was used for estimation Furosemide in tablet [11], Some researchers depending on diazotized method to assay FSD [12], liquid chromatography were reported for estimation FSD in plasma and urine samples [13,14], as well as polarographic method [15]. Other method was based on using schiff's bases to estimate FSD spectrophotometrically [16]. Also, liquid-liquid extraction and high-performance liquid chromatography were used for estimation FSD [17,18]. Spectrophotometric methods adopted different reactions for the determination of FSD in pharmaceutical dosage forms [19-22], finally, reverse-phase high-performance liquid chromatography [23], and flow injection with HPLC [24], as well as HPLC method were used in the estimation of FSD [25-27].

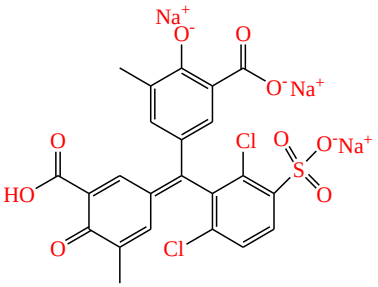
In this paper, three an organic dyes have been used for estimating FSD, chromazurol S, xylenol orange and bromophenol blue, FSD was bleaching both of xylenol orange and bromophenol blue, where, chromazurol S was differed from them and increase the absorbance intensity as increasing FSD concentration, depending on this principle, FSD was assayed in three methods, method A bleaching bromophenol blue with increasing FSD amount at pH 3.63, as well as method B with xylenol orange at pH 4.72, While method C based on charge transfer reaction between chromazurol S and FSD at pH 4.77 with increasing FSD amount. So that, the organic dye used in method A is bromophenol blue which is chemically and traditionally named as 3′,3″,5′,5″-tetra bromophenolsulfonphthalein, albutest respectively was prepared by the slowly addition of an excess bromine to a hot phenolsulfonphthalein in glacial acetic acid solution [28], Xylenol orange is an organic reagent used as indicator for metal titration, Xylenol orange is the traditionally name of 2,2′,2″,2‴-{(1,1-Dioxo-2-benzoxathiole-3,3 (1H)-diyl) bis [(6-hydroxy-5-methyl-3,1-phenylene) methylenenitrilo]}tetra acetic acid [29]. Trisodium 5-[(E)-(3-carboxy-5-methyl-4-oxocyclohexa-2,5-dien-1-ylidene)(2,6-dichloro-3-sulfonatophenyl)methyl]-3-methyl-2-oxidobenzoate is the chemical name for



Bromophenol blue



Xylenol orange



Chromazurol S

chromazurol S, this reagent was widely used for determination of cations as well as medications in direct and indirect ways [30].

2. EXPERIMENTAL

2.1 Apparatus

The final spectrum of FSD was measured and drawn using JASCO V – 630 double-beam computerized UV-Visible spectrophotometer, for all spectrophotometric measurements, 1 cm matched cell was used, and HANA pH meter used for reported pH readings.

2.2 Analytical Chemicals

All chemicals used were of the purest analytical grade.

2.3 Preparation of Furosemide from Tablets

Three different brands of pharmaceutical preparations were used for furosemide, where 10 tablets (each tablet contains 40 mg) were ground into a very fine powder, then weighed precisely about 0.01 g of the powder, this quantity was then dissolved in methanol, filtered and completed the volume of filtration with methanol mixed with warm distilled water at a ratio of 1:1 in a 100 ml volumetric flask.

3. DISCUSS THE EXPERIMENTAL RESULTS

100 µg of FSD in a final volume of 25 mL was used to study the experimental optimal conditions

3.1 Study of Optimum Conditions

In this research paper, the optimal conditions suitable for the formation of the colored complex of furosemide were studied and selected.

3.2 Selected the Optimum Medium of the Reaction

In order to choose the most appropriate type of acids and in the optimum quantity among sulfuric, acetic and hydrochloric acids, by studying the effect of adding different quantities to each type of these acids to determine the acidity function most appropriate for the three methods for estimating furosemide, a (0.1-3.0) of these acids was chosen with a concentration of 0.1 M as shown in Table 2.

The practically obtained results and illustrated in Table 1, that the addition of any type at any amount of all acids did not have a beneficial effect, therefore, this study was excluded from the subsequent experiments. Depending on this fact, the pH value of method A, B, and C in the absence of any quantity of acids or bases were 3.58, 4.51 and 4.77 respectively, so that, these pH value have adopted for the subsequent experiment.

Table 1. Preparation of chemical materials

Chemical materials	Manufactured company	Weight, g	Solvent in final volume 100 mL	Concentration
FSD	SDI, Iraq	0.01	Ethanol [31]	100 µg/ ml
Bromo phenol blue	Hopkin and williams	0.6699	Distilled water	0.01 M
Xylenol orange	Fluka	0.7585	Distilled water	0.01 M
Chrom azurol S	Fluka	0.0605	Distilled water	0.001 M

Table 2. Study the optimum medium for methods A, B and C

ml of 0.1M acids	Method A Absorbance			Method B Absorbance			Method C Absorbance		
	H_2SO_4	HCl	CH_3COOH	H_2SO_4	HCl	CH_3COOH	H_2SO_4	HCl	CH_3COOH
0.0	0.645			0.521			0.364		
0.1	0.134	0.168	0.174	0.109	0.104	0.142	0.164	0.138	0.144
0.3	0.119	0.126	0.159	0.098	0.094	0.128	0.141	0.112	0.134
0.5	0.094	0.109	0.124	0.072	0.071	0.110	0.119	0.094	0.101
0.7	0.086	0.099	0.093	0.062	0.057	0.089	0.089	0.080	0.089
1	0.064	0.078	0.076	0.041	0.042	0.070	0.065	0.068	0.072
1.5	0.051	0.059	0.044	0.024	0.028	0.053	0.031	0.051	0.059
2	0.033	0.043	0.028	0.013	0.014	0.038	0.017	0.037	0.039

3.3 Effect of Dye Quantity

The experimental results which were depending on the values of the correlation coefficient and absorbance values were considered the best factor to choose the optimum amount of dyes in the three proposed methods. 3 mL, 2 mL of 0.01 M and 2 mL of 0.001 M, have been selected as an optimum amount of these three dyes with correlation coefficient equals to (0.99863, 0.98972 and 0.9992) for method A, B, and C respectively.

3.4 Effect of Various Kinds of Surfactants

In many cases, the addition of any type of surfactant of different types may not lead to a shift in the wavelength or an improvement in the intensity of absorption, as happened in this study.

It was noted from the practical results that adding all types of surfactants (sodium dodecyl sulfate as a surfactant) Anionic, cetyltrimethylammonium bromide, cetylpyridinium chloride as cationic surfactants and non-ionic Triton X-100) to the staining regimen had no obvious effect either in increasing the absorption intensity or leading to the wavelength shift to higher values. Therefore, this study was not adopted in subsequent experiments.

3.5 Studying Order of Addition

The interaction components of the three methods do not exceed the drug and the dye, so there are no more than two sequences to study that lead to the same result, adding the dye to the drug or vice versa did not have a clear effect, so the addition of the dye to the furosemide drug was adopted in this study.

3.6 Studying Stability Period

The time required for the formation of the colored potion between FSD and the three dyes was studied, as it was found from the practical results that the color formed instantaneously and remained stable for more than 72 hours with a high stability of the three methods under optimal conditions, meaning that the colored compound was developed immediately and remains at a maximum and consistently and very stable more than 72 hours. Fig. 1 show a part of stability for this study for these three methods.

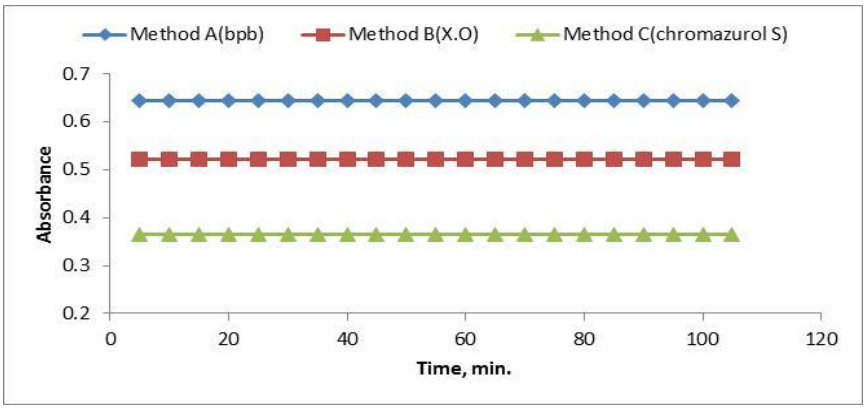


Fig. 1. Stability of the product coloured complex

3.7 Beer's Law, Molar Absorption and Sensitivity

The standard curve of the proposed spectroscopic methods has been studied by adding different amounts ranging between (10-800), (25-800) and (20-800) for each method A, B and C respectively, then adding dyes and dilution to the mark with distilled water and after shaking the bottles. The absorbance was measured at the specified wavelength at 591, 583, and 525 nm, complied with Beer's law over the f and ppm of FSD While the molar absorbance was 1.4×10^4, 2.1×10^4 , 1.57×10^4 $l.mol^{-1}.\ cm^{-1}$ and 0.0157, 0.0236 and 0.0210 $\mu g.cm^{-2}$, as shown in Figs. 2, 3, and 4 respectively.

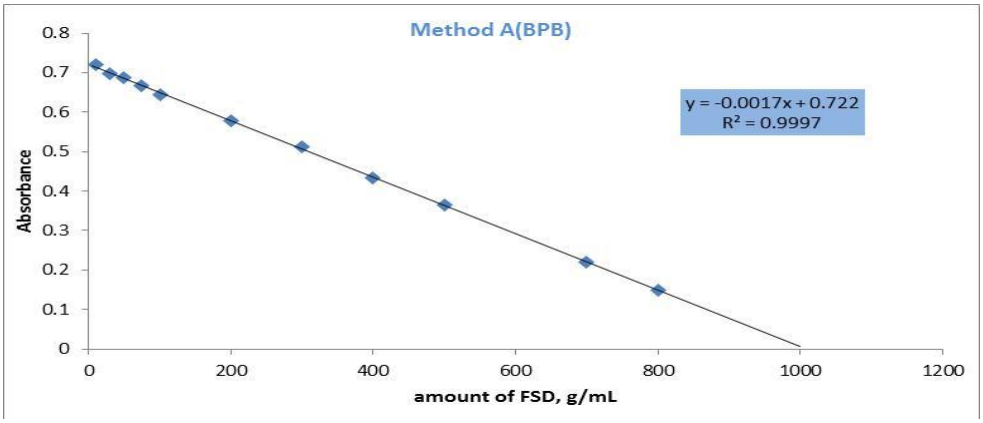


Fig. 2. Calibration curve for the first method

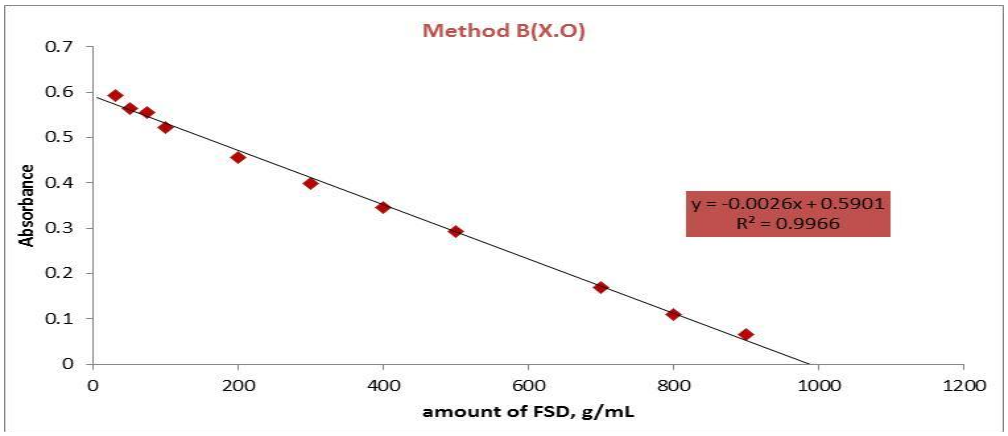


Fig. 3. Calibration curve for the second method

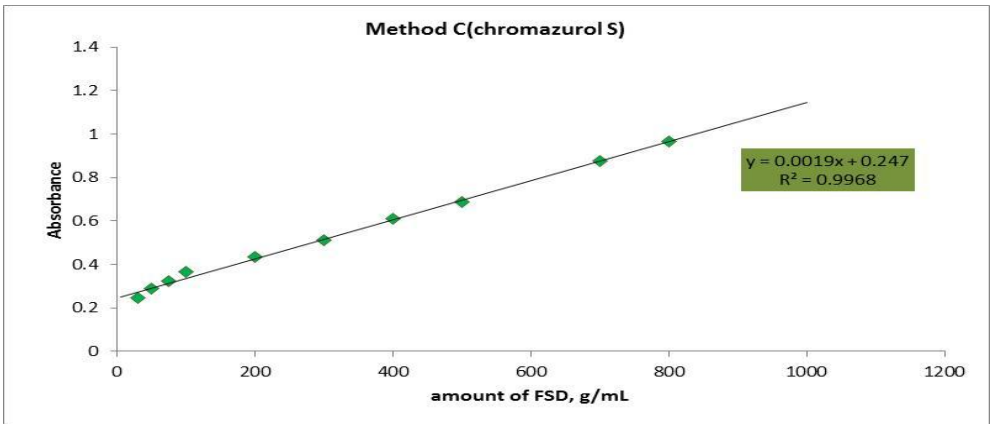


Fig. 4. Calibration curve for the third method

3.8 Absorption Spectra

Depending on the optimum conditions, the absorption spectrum of FSD was studied, as shown in Figs. 5, 6 and 7 which were indicate that the sample solution shows maximum absorption at 591, 583 and 525 nm for the three methods, respectively.

3.9 Accuracy and Precision

In order to verify the selectivity and efficiency of the proposed methods for FSD estimation, 100µg of FSD were determined using ten measurements for each method as shown in Fig. 8 which is illustrated that these three methods were almost reliable.

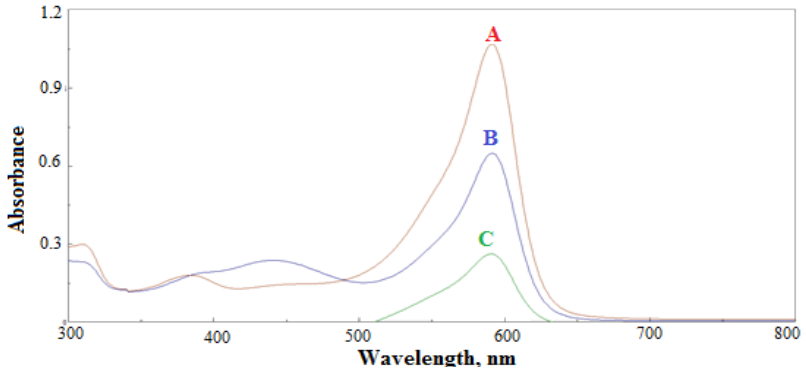


Fig. 5. Final spectrum of 100 µg FSD for the method A, measured against Blank (B), Distilled water (A) and Blank measured against distilled water (C)

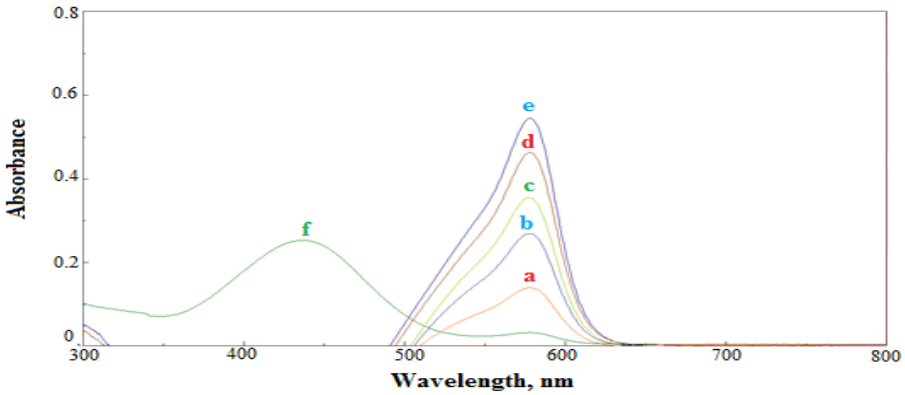


Fig. 6. Final spectrum of (700, 500, 300, 200 and 100) µg FSD for the 2nd method meaured against Blank (a, b, c, d and e), and Blank measured against distilled water (f).

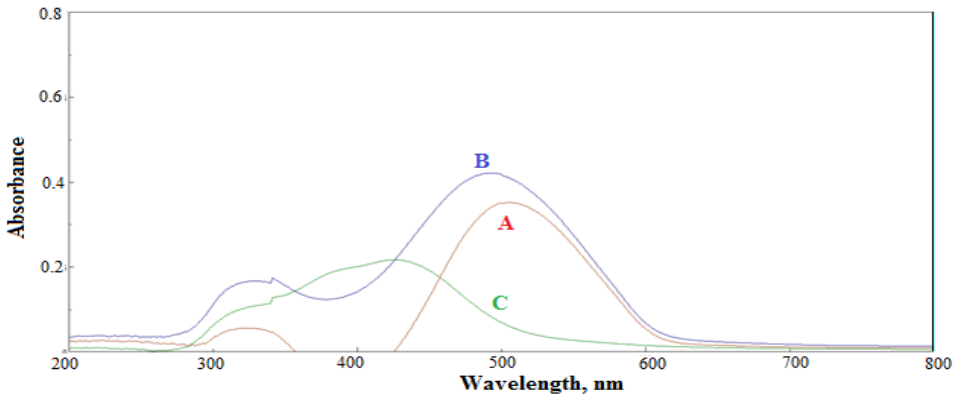


Fig. 7. Final spectrum of 100 µg FSD for the 3rd method measured at Blank (A), Distilled water (B) and Blank measured against distilled water (C)

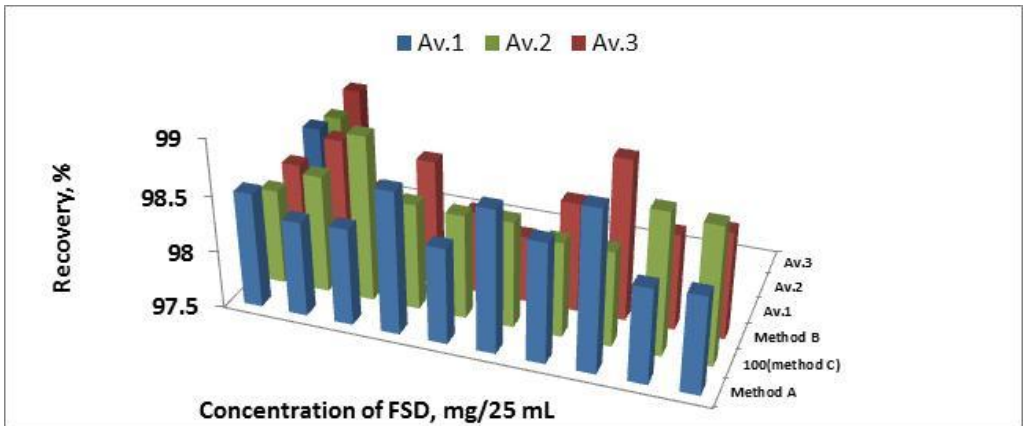


Fig. 8. Accuracy and precision

3.10 Mole Ratio

The determination of the interaction ratio between furosemide drug and the three dyes was studied by preparing equal concentrations for both drug and dyes, then taking volumes ranging from (0-5) mL of FSD, corresponding to (5-0) mL of each of the three dyes. Fig. 11 shows the result of using Job's method to study the reaction ratio in each method, so that, reaction ratio of FSD to bromophenol blue is 1:2, and 1:1 between FSD to xylenol orange dye, while the ratio of FSD to chromazurol S is 1:2 as shown in the Fig. 9.

3.11 Effect of Foreign Materials

This study was conducted by adding a number of potential substances present and use in pharmaceutical preparations, with concentrations of up to 1000 µg/mL. The results listed in Table 3 showed that the studied excipients do not seriously interfere in the determination of FSD in pharmaceutical preparations using the three proposed methods.

3.12 Application of the Method

The three proposed methods have been satisfactorily applied for the estimation of FSD in pharmaceuticals and the results are shown in Table 4.

It is noted from the results listed in Table 5 that the calculated value of the t-test measured at 95% confidence level and for five degrees of freedom (N1 + N2-2 = 5) did not exceed the theoretical values for that when compared with the theoretical values established in the references [32].

3.13 Comparison of the Present Method

Table 6 shows some of the analytical variables measured for the current methods and their comparison with the spectroscopic methods proven in the references for the estimation of FSD.

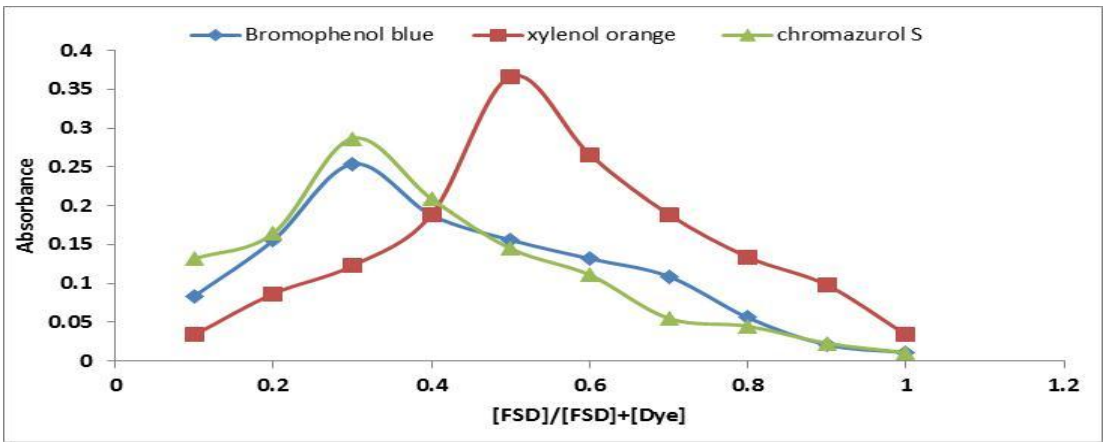


Fig. 9. Job's plot for methods A, B and C

Table 3. Effect of interferences

Interferences	Recovery (%)/100 µg of FSD added								
	Method A			Method B			Method C		
	100	500	1000	100	500	1000	100	500	1000
Acatia	99.34	99.67	98.82	99.85	98.93	98.99	99.92	99.93	99.41
Lactose	99.87	99.99	99.07	99.74	98.97	98.95	99.89	98.98	98.76
Sorbitol	99.89	98.97	98.73	98.69	98.87	98.90	99.86	99.93	98.93
Glucose	99.51	99.39	98.22	98.94	98.99	98.95	99.93	98.92	98.80
Menthol	98.97	98.98	98.97	98.92	98.89	98.94	99.86	99.89	98.82
Starch	99.39	99.34	99.56	99.89	98.87	98.97	99.90	98.84	98.79

Table 4. Determination of FSD

Drug	µg of FSD present/25 ml		µg of FSD measured/25 ml	R*, %	R.E *, %
Furosemide/tablets 40mg/tab	Method A	100	99.85	99.85	±0.5291
	Method B		99.81	99.81	±0.2902
	Method C		100.27	100.27	±0.2889
Octosemide/tablets 40mg/tab	Method A	300	299.34	99.79	±0.4071
	Method B		299.29	99.78	±0.3237
	Method C		300.71	100.23	±0.2691

**For 5 determination*

Table 5. The value of t-test

drug	t-test	Tabulated value of t-test
Furosemide/tablets 40mg/tab	±1.854	± 2.571
octosemide/tablets 40mg/tab	± 1.497	

Table 6. Comparison of the method

Analytical parameters	Present method			Literature method [32]	
	Method A	Method B	Method C	Method A	Method B
Reaction	Bleaching	Bleaching	Charge transfer	Oxidation with Bleaching	Oxidation with Bleaching
pH	3.58	4.51	4.77	---	----
λ_{max} (nm)	591	583	525	612	526
Reagent	Bromophenol blue	Xylenol orange	Chromazurol S	Xylene cyanol FF	Safranin O
Correlation coefficient	0.9997	0.9966	0.9968	0.9992	0.9996
Beer's law range (ppm)	0.4-32	1-32	0.8-32	20-30	6-16
Molar absorption ($l.mol^{-1}.cm^{-1}$)	1.4×10^4	2.1×10^4	1.57×10^4	1.16×10^4	2.02×10^4
R.S.D. (%)	±0.5291 to ±0.4071	±0.3237 to ±0.2902	±0.2889 to ±2691	0.099	1.8345
Color of the product	Red	Red	Red	Blue	Red
Application of the method	Pharmaceutical preparations	Pharmaceutical preparations	Pharmaceutical preparations	Pharmaceutical preparations	Pharmaceutical preparations

4. CONCLUSION

Three spectroscopic methods are proposed based on the reaction of charge transfer and shortening of the dye using bromophenol blue in method 1, xylenol orange in method 2, and chromazorol S in rapid, sensitive and inexpensive methods that do not require

expensive devices or any temperature control or any extraction process. Good recovery values for FSD are achieved upon successful application of the proposed methods for determining FSD in some of its pharmaceutical preparations.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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