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Rapid titrimetric and spectrophotometric determination of frusemide (furosemide) in formulations using bromate-bromide mixture and methyl orange

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Two methods based on titrimetric and spectrophotometric techniques using bromate-bromide mixture and methyl orange as reagents are described for the determination of frusemide in bulk drug and formulations. In titrimetry, an acidified solution of frusemide is titrated directly with bromate-bromide mixture using methyl orange as indicator. Spectrophotometry entails adding a fixed and known amount of bromate-bromide mixture to an acidified solution of drug and determination of the residual bromine by reacting with a fixed amount of methyl orange and measuring the absorbance at 520 nm. The amount of bromine reacted corresponds to drug content. The quantification in titrimetry is based on a 1:0.333 reaction stoichiometry (Frusemide: $KBrO_3$) and the method is applicable over 2-20 mg range. In spectrophotometry, the calibration graph is found to be linear over 0.25-3.50 μg mL^{-1} range with a molar absorptivity of 6.11×10^4 L mol^{-1} cm^{-1} and a Sandell sensitivity of 5.18 ng cm^{-2}. The limits of detection and quantification are calculated to be 0.07 μg mL^{-1} and 0.24 μg mL^{-1}, respectively. The statistical evaluation of the methods was examined by determining the intra-day and inter-day precision. The methods were applied to the determination of frusemide in tablets and the results were found to agree well with the declared amounts. The accuracy and reliability of the proposed methods were further ascertained by parallel determination by a reference method and by recovery studies using standard addition technique.

Keywords: Frusemide, determination, titrimetry, spectrophotometry, bromate-bromide, methyl orange.

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Frusemide (FRU) is an anthranilic acid derivative extensively used for its diuretic effect in the treatment of edema associated with pulmonary, cardiac, hepatic and renal disease[1], and of hypertension accompanied by fluid retention or impaired renal failure[2]. Because of its predominant action on the loop of Henle and the marked dieresis it can produce, this compound is often designated as a loop diuretic and high ceiling diuretic[1].

Numerous methods have been reported for the determination of FRU in pharmaceutical samples. Most of the methods developed are based on different chromatographic techniques such as high performance liquid chromatography (HPLC)[3-8,] liquid chromatography-mass spectrometry(LC-MS)[9], micellar liquid chromatography(MLC)[10] and thin layer chromatography(TLC)[11]. Several other techniques such as differential pulse voltammetry(DPV)[12], proton nuclear magnetic resonance spectroscopy(H^1NMR)[13], UV-spectrophotometry[14], derivative UV-spectrophotometry[15] and difference UV-spectrophotometry[16,17] have been applied for the assay of FRU in bulk drug and formulations.

The literature on the titrimetric assay of FRU is scarce. Two indirect titrimetric methods[18,19] employing vanadium(V) as the oxidimetric reagent, involve boiling the reaction mixture for 20-30 min before back titrating the unreacted vanadium(V). Yang *et al.*[20] have reported an oscillopotentiometric titration with lithium methoxide as titrant with use of bimetallic electrodes in dimethyl formamide medium. But, each determination requires 200 mg of FRU. A coulometric titration[21] with electrogenerated chlorine using methyl orange as indicator has also been suggested for the assay of FRU.

One widely employed technique for the determination of FRU in pharmaceuticals has been the visible spectrophotometry and procedures based on such diverse reactions as binary complexation[22-24], ternary complexation[25], ion-pair complexation[26,27], charge-transfer complexation[28], derivatisation[29], redox[30], diazocoupling[31] and oxidative coupling[32,33] are found in the literature. However, most of the

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reported methods suffer from disadvantages such as poor sensitivity, heating step, extraction step or use of expensive and undesirable chemicals (Table 1).

Bromate-bromide mixture in combination with methyl orange has been widely used for the titrimetric and spectrophotometric determination of numerous pharmaceuticals[34-44]. In this work, this combination has been evaluated as a reagent for the titrimetric and spectrophotometric determination of FRU in pharmaceutical samples. In titrimetry, FRU is titrated directly with bromate-bromide solution in acid medium using methyl orange as indicator. Spectrophotometry involves adding a fixed and known amount of the mixture to FRU solution under acidic conditions followed by estimation of the residual *in situ* generated bromine based on its bleaching action on methyl orange. The developed methods offer the advantages of speed, simplicity, sensitivity, and accuracy and precision.

Experimental Procedure

Titrimetry

All chemicals used were of analytical reagent grade and double distilled water was used to prepare all solutions. A bromate-bromide mixture (5 mM $KBrO_3$-50 mM KBr) was prepared by dissolving 0.835 of $KBrO_3$ (Qualigens Fine Chem, India) and 6 g of KBr (S. d. Fine Chem, Mumbai, India) in water and diluting to 1 L in a volumetric flask and used for titrimetric work. Methyl orange indicator (0.5 %) was prepared by dissolving 50 mg of dye (S. d. Fine Chem. India) in 10 mL of water. Hydrochloric acid (2 M) was prepared by diluting 177 mL of concentrated acid (S. d. Fine Chem. India, sp. gr. 1.18) to 1 L with water.

Spectrophotometry

A stock solution of $KBrO_3$- KBr equivalent to 1000 μg mL^{-1} $KBrO_3$ was prepared by dissolving accurately

Table 1—Comparison of the existing spectrophotometric methods with the proposed method for frusemide

S No.	Reagent(s) uesd	Linear range, μg mL^{-1} (ε, L mol^{-1} cm^{-1})	Remarks	Ref.
1	Palladium(II) chloride	79-1030	Less sensitive	22
2	Palladium(II) chloride	6-127	Uses Fia assembly where the reactor is maintained at 55 °C	23
3	Iron(III)	30 (LOD)	Measurement at 410 nm. Requires strict pH control; less sensitive	24
4	Fe(III)-SCN^-	400-4300	Involves extraction with $CHCl_3$; requires strict pH control; least sensitive	25
5	Methyl violet	20-120 ($\varepsilon = 3.4 \times 10^4$)	Requires strict pH control; involves extraction with $CHCl_3$	26
6	Amethyst violet	—	Requires strict pH control; involves extraction with $CHCl_3$	27
7	Tetracyanoquinodimethane	0.5-8.0 ($\varepsilon = 4.56 \times 10^4$)	Requires strict pH control; uses acetonitrile throughout	28
8	Sodium naphtha quinone sulphonate	1.2 (LOD)	Requires strict pH control and heating at 70 °C for 30 min; involves extraction with isoamyl alcohol	29
9	Molybdophosphoric acid	5-200 ($\varepsilon = 2.16 \times 10^3$)	Involves heating at 98 °C for 20 min	30
10	$NaNO_2$-NNED*	2-10	Involves heating at 60 °C for 30 min and cooling at 15 °C; 15 min contact time	31
11	Iron(III)-MBTH*	1-10 ($\varepsilon = 1.76 \times 10^4$)	Involves heating at 85 °C for 5 min	32
12	CAT-NNDPD*	5-60 ($\varepsilon = 2.64 \times 10^4$)	Involves heating at 70 °C for 10 min	33
13	BrO^-_3-Br^-/methyl orange	0.25-3.5 ($\varepsilon = 6.11 \times 10^4$ LOD = 0.07)	No heating step; highly sensitive	Present method

*NNED=N-1-naphthalethylenediammonium chloride
MBTH=3-methyl-2-benzothioazolinone-hydrazone hydrochloride
CAT=Chloramine-T
NNDPD=p-N, N-dimethyl phenylnediamine dihydrochloride
LOD=Limit of detection

weighed 100 mg of $KBrO_3$ and a large excess (~1 g) of KBr in water and diluting to 100 mL in a calibrated flask. This was diluted stepwise to get 10 μg mL^{-1} $KBrO_3$ solution. A 500 μg mL^{-1} methyl orange solution was prepared by dissolving 52.4 mg of dye in water and diluting to 100 mL in a calibrated flask, and filtered. This was diluted 10 fold to get 50 μg mL^{-1} working solution. Hydrochloric acid (5 M) was preapred by appropriate dilution of concentrated acid.

Pharmaceutical grade FRU was obtained from M/s Hoechest Morrison Roussel Ltd, Mumbai, India, and was used as such. A stock standard solution containing 2 mg mL^{-1} FRU was prepared by dissolving 500 mg of pure drug in 125 mL of glacial acetic acid and diluting to the mark with water in a 250 mL calibrated flask, and used in titrimetric assay. The stock solution (2000 μg mL^{-1} FRU) was diluted stepwise with 1:1 acetic acid to 10 μg mL^{-1} solution for spectrophotometric determination.

Titrimetric assay

A 10 mL aliquot of pure drug solution containing 2-20 mg of FRU was accurately transferred into a 100 mL titration flask, 10 mL of 2 M HCl was added and titrated with bromate-bromide mixture (5 mM w. r. t. $KBrO_3$) using 2 drops of methyl orange indicator till the disappearance of the indicator colour. A blank titration was performed and the volume of titrant was subtracted from the volume required for drug solution titration. The amount of FRU in the measured aliquot was calculated from:

$$\text{Amount (mg)} = \frac{VM_wR}{0.333}$$

where V = volume of bromate-bromide consumed, mL

M_w = relative molecular mass of drug

R = molarity of bromate-bromide mixture w. r. t. $KBrO_3$.

Spectrophotometric determination

Different aliquots (0.25-3.50 mL) of 10 μg mL^{-1} FRU solution were accurately measured into separate 10 mL calibrated flasks and the total volume was adjusted to 5 mL by adding water. To each flask was added 1 mL each of 5 M HCl and bromate-bromide mixture (10 μg mL^{-1} w.r. t. $KBrO_3$). The flasks were stoppered, contents mixed well and let stand for 10 min with occasional shaking. Then, 1 mL of 50 μg mL^{-1} methyl orange solution was added to each flask and diluted to the mark with water. The absorbance of each solution was measured at 520 nm against a reagent blank after 5 min. A calibration curve was prepared by plotting the absorbance versus concentration of FRU. The concentration of the unknown was read from the calibration graph or calculated from the regression equation derived from the Beer's law data.

Method for formulations

Twenty tablets were accurately weighed and ground into a fine powder with agate pestle and mortar. An amount of the powdered tablet containing 100 mg of FRU was accurately weighed into a 100 mL calibrated flask, 60 mL of glacial acetic acid added and shaken thoroughly for about 20 min. Then, the volume was made up to the mark with water, mixed well and filtered using a Whatman No.42 filter paper. First 10 mL portion of the filtrate was discarded and 10 mL of the subsequent portion was titrated as described previously. The tablet extract (1000 μg mL^{-1}) was appropriately diluted to obtain 10 μg mL^{-1} concentration and a suitable aliquot was subjected to analysis by spectrophotometry. An accurately measured aliquot of injection solution equivalent to 100 mg of FRU was transferred into a 100 mL calibrated flask, 15 mL glacial acetic acid added and diluted to the mark with water. The assay was completed as described above.

Results and Discussion

Optimisation of reaction conditions

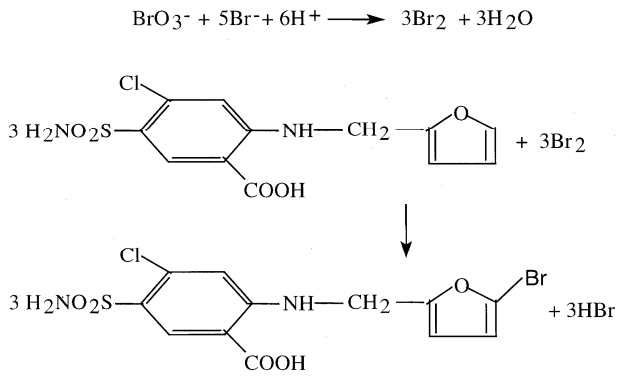
Tritimetry

The quantitative nature of the reaction between FRU and *in situ* generated bromine was checked by titrating 2-20 mg of drug to a methyl orange end point. In the range studied, the reaction stoichiometry was found to be 3:1 (FRU: $KBrO_3$) which can be represented by scheme 1.

The reaction was carried out in HCl medium and the reaction stoichiometry was found to be unaffected when 5-20 mL of 2 M HCl was used in a total volume of 30-40 mL. The linear relationship between the drug amount and the titration end point is apparent from the calculated correlation coefficient of 0.9986 obtained by the best fit line via least squares treatment.

Spectrophotometry

In the proposed method, different amounts of drug were added to a fixed amount of bromate-bromide



Scheme 1

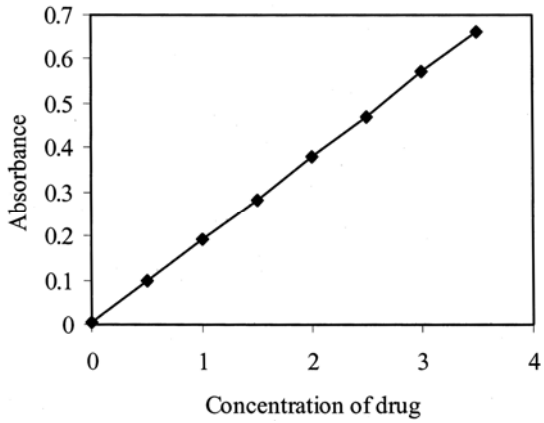


Fig. 1—Linearity curve

mixture in HCl medium and after the reaction was ensured to be complete, the residual bromine was determined by treating with a fixed amount of methyl orange dye, and measuring the absorbance at 520 nm. The absorbance was found to be linearly dependent on the concentration of FRU.

FRU, when added in increasing amounts to a fixed and known excess amount of *in situ* generated bromine, consumes the latter, and there will be a concomitant decrease in the amount of bromine. When a fixed amount of methyl orange is added to decreasing amounts of bromine, a concomitant increase in the dye concentration results which is reflected in the proportional increase in the absorbance at 520 nm (Fig.1).

Preliminary experiments were performed to fix the upper limit of methyl orange concentration that could be spectrophotometrically determined in acid medium and this was found to be 5 μg mL^{-1}. A bromate concentration of 1 μg mL^{-1} in the presence of a large excess of bromide was found to quantitatively bleach the red colour due to 5 μgmL^{-1} methyl orange. To determine the concentration range of applicability, therefore, different concentrations of FRU were reacted with 1 mL of 10 μg mL^{-1} bromate in acid medium and in the presence of a large excess of bromide followed by determination of residual bromine as described earlier.

Hydrochloric acid was the medium of choice for the bromination step as well as the determination of the residual bromine with the dye. One mL of 5 M HCl in a total volume of 5 mL was convenient for the bromination step which was complete in 5-10 min and the same concentration was employed for the determination of the dye although HCl concentration of 0.125-1.25 M did not affect the absorbance of the dye colour. Contact time of 10 min is not critical and any delay up to 30 min in adding the dye had no effect on the absorbance. A 5-min standing time was necessary for the complete bleaching of the dye colour by the residual bromine. The absorbance of dye was stable for several hours even in the presence of the reaction product.

Analytical data

Beer's law was obeyed over the concentration range 0.25-3.50 μg mL^{-1}. The apparent molar absorptivity and Sandell sensitivity were calculated to be 6.11×10^4 l mol^{-1} cm^{-1} and 5.18 ng cm^{-2}, respectively. The linear plot gave the regression equation:

$$A = 3.86 \times 10^{-3} + 0.188C \ (r = 0.9944, n=8)$$

where A is the absorbance and C concentration in μg mL^{-1}. The detection and quantification limits were calculated from the standard deviation of absorbance measurements obtained from a series of seven blank solutions. The limit of detection (K=3) and the limit of quantification (K=10) were established according to the IUPAC definition[45] and were calculated to be 0.07 and 0.24 μg mL^{-1}, respectively.

Validation of methods

Accuracy and precision

To establish the accuracy and precision of the proposed methods, pure drug at three different levels (within the working limits) was determined, each determination being repeated seven times. The relative error (%) which is a measure of accuracy and RSD (%) a measure of precision are summarised in Table 2 and reveal the high accuracy and precision of the methods. For a better picture of reproducibility on

a day-to-day basis, a series was run in which the standard drug solution at three levels was analyzed each day for five days. The day-to-day RSD values were in the range of 1.84-2.35 % and represented the best appraisal of the methods in routine use.

Determination of FRU in tablets and injections

The proposed methods were applied to the determination of FRU in some representative tablets and injections which were commercially available in the local market. The drug content of same batch tablets and injections was also determined by the official method and the results are presented in Table 3. It is clear from the results that there is close agreement between the results obtained by the proposed methods and those of the reference method.

The results were also compared statistically by Student's *t*-test for accuracy and a variance ratio *F*-test for precision with those of the reference method at 95 % confidence level. The calculated *t*- and *F*-values (Table 3) did not exceed the tabulated values (t = 2.77, F=6.39) for four degrees of freedom indicating that there was no significant difference between the proposed methods and the reference method in respect of accuracy and precision.

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre analyzed tablet powder and injection

Table 2—Evaluation of accuracy and precision of methods

Titrimetry					Spectrophotometry				
Drug taken, mg	Drug found, mg	RE, %	RSD, %	Range of error$^{\varphi}$, %	Drug taken, µg	Drug found, µg	RE, %	RSD, %	Range of error$^{\varphi}$, %
5.0	5.08	1.60	1.87	± 1.80	10.0	10.18	1.80	1.78	± 1.71
10.0	9.92	0.80	0.36	± 0.35	20.0	19.84	0.80	1.26	± 1.21
15.0	14.72	1.87	0.55	± 0.53	30.0	29.86	0.93	2.12	± 2.04

*Mean value of seven determinations
$^{\varphi}$at 95 % confidence level for 6 degrees of freedom
RE-Relative error
RSD-Relative standard deviation

Table 3—Results of assay of formulations

Formulation and brand name*	Label claim, mg/tablet or mg/mL	**Found (% label claim ± SD)		
		Reference method	Titrimetric method	Spectrophoto-metric method
Frusenex[a] tablets	40	101.36 ± 0.58	100.96 ± 1.04 t = 1.28 F= 3.21	102.64 ± 1.46 t = 1.98 F = 6.33
	100	99.85 ± 0.62	102.14 ± 1.28 t = 3.80 F = 4.26	101.32 ± 0.94 t = 2.97 F = 2.30
Lasix tablets[b]	40	102.66 ± 0.74	103.59 ± 0.28 t = 3.12 F = 6.98	101.42 ± 1.84 t = 1.52 F = 6.18
Lasix injections[b]	10	98.34 ±1.26	100.03 + 0.84 t = 2.54 F = 2.25	99.75 ± 2.36 t = 1.70 F = 3.51
Salinex tablets[c]	40	103.66 ± 0.82	105.28 ± 1.64 t = 3.50 F = 4.0	102.76 ± 1.03 t = 1.53 F = 1.58

*Marketed by: a. Geno Pharmaceuticals Ltd; b. Hoechst Morrison Roussel Ltd;
c. Indian Drugs and Pharmaceuticals Ltd.,
**Mean of five determinations
Tabulated t-value at 95% confidence level is 2.77
Tabulated F-value at 95% confidence level is 6.39

Table 4—Results of recovery study by standard-addition technique

Formulation studied	Titrimetry				Spectrophotometry			
	Amount in formulation, mg	Pure drug added, mg	Total* found, mg	Recovery of pure drug added, %	Amount in formulation, µg	Pure drug added, µg	Total* found, µg	Recovery of pure added, µg
Frusenex tablets (40 mg)	3.03	5.0	8.16	102.64	5.13	10.0	15.59	104.63
	3.03	10.0	13.35	103.18	5.13	15.0	20.56	102.85
	3.03	15.0	18.24	101.39	5.13	20.0	25.74	103.64
Frusenex tablets (100 mg)	3.06	5.0	8.08	100.38	5.07	10.0	15.45	103.66
	3.06	10.0	13.23	101.66	5.07	15.0	20.93	105.72
	3.06	15.0	18.66	104.03	5.07	20.0	25.44	101.84
Lasix injections (10 mg)	3.00	5.0	7.93	98.62	4.99	10.0	15.15	101.62
	3.00	10.0	12.79	97.88	4.99	15.0	19.34	98.31
	3.00	15.0	18.04	100.24	4.99	20.0	24.84	99.24

*Mean value of three trials

solutions were spiked with pure FRU at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The recovery of pure drug added was quantitative (Table 4) and revealed that coformulated substances such as talc, starch, gum acacia, lactose, sodium alginate, magnesium stearate, calcium gluconate and calcium dihydrogenorthophosphate did not interfere in the determination.

Conclusions

Two useful methods for the determination of frusemide using bromate-bromide mixture and methyl orange have been developed and validated. The titrimetric method is simple to perform unlike the previously reported methods which require a heating step and is applicable over a long dynamic range (2-20 mg). The spectrophotometric method is also easier and cheaper to perform compared to many existing methods for frusemide. A significant advantage of the spectrophotometric method is its high sensitivity which surpasses that of all the previously reported methods and is free from either heating or extraction step. The proposed methods do not entail any stringent experimental variables, which affect the reliability of the results. The methods have been successfully applied to the determination of frusemide in formulations with the percent recoveries in the range of 99.75-105.28 indicating the non-interference from common additives and excipients.

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