Indian Journal of Chemical Technology
Vol. 17, March 2010, pp. 150-153

Spectrophotometric determination of frusemide by its oxidation with ceric ammonium sulphate

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Received 3 August 2009; revised 7 December 2009

A simple and sensitive spectrophotometric method is described for the determination of frusemide (FRU). The method is based on the reaction of FRU with measured excess of ceric ammonium sulphate (CAS) followed by the determination of unreacted oxidant using xylene cyanol FF (XC) (Method A) and safranin O (SAF) (Method B). The reaction mixture exhibited maximum absorbance at 612 nm (Method A) and 526 nm (Method B). The calibration graph was linear from 20.00 to 30.00 µg mL^{-1} and 6.00 to 16.00 µg mL^{-1} for methods A and B respectively. The apparent molar absorptivity and Sandell's sensitivity for method A and B were calculated to be 1.160×10^4 L $mol^{-1}cm^{-1}$, 2.025×10^4 L mol^{-1} cm^{-1} and 2.82×10^{-3} µg cm^{-2}, 0.0163 µg cm^{-2} respectively. The method has been applied for the determination of frusemide in pure and dosage forms.

Keywords: Frusemide determination, Oxidation, Ceric ammonium sulphate, Spectrophotometry

Frusemide or furosemide (4-chloro-N-furfuryl-5-sulfamoyl-anthranilic acid), a sulfonamide, is an antibacterial agent (Fig. 1). Frusemide is often classified as a loop diuretic due to its predominant action in the nephron, where the drug interferes with the tubular re-absorption of sodium on Henle's loop[1]. The renal excretion of ions is not limited to sodium and chloride, but it may also influence potassium, magnesium, calcium and, to a lesser extent, hydrogen carbonate ions[2]. In the clinical practice, the effects of frusemide are applied in the treatment of edema associated with pulmonary, cardiac, hepatic and renal diseases, and of hypertension accompanied by fluid retention or renal failure[3-7].

Frusemide is frequently administered as oral tablets, or through intravenous injections. More recently, aerosolized frusemide has evidenced a new mechanism of action[8]. Inhaled furosemide provides a protective effect against respiratory infections by acting on the pulmonary system without causing diuresis.

Several spectrophotometric[9-15] methods have been reported for the determination of frusemide in bulk, pharmaceutical dosage forms, and/or biological fluids. The present investigation deals with a simple method for the determination of frusemide using ceric ammonium sulphate and xylene cyanol FF and safranin O. The method is sensitive, accurate and precise and gave satisfactory results when applied to formulations containing frusemide.

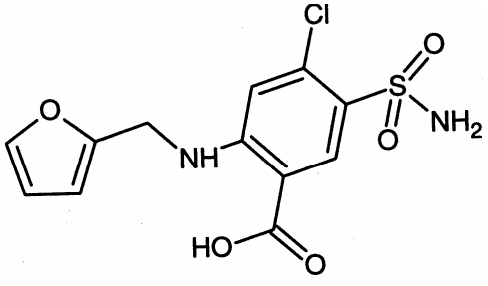
Experimental Procedure

Apparatus

A Shimadzu UV-2550 UV-VIS Spectrophotometer with 1 cm matched quartz cells was used for absorbance measurements.

Materials and Reagents

All reagents used were of analytical grade. All solutions were prepared using distilled water. Ceric ammonium sulphate (CAS) (0.3%), H_2SO_4 (2 N), xylene cyanol FF (XC) (0.05%) and safranin O (SAF) (0.01%) of AR grade were used. CAS (0.3%) was prepared by dissolving 0.75 g of CAS in 2 N H_2SO_4 and diluting to the mark with distilled water in a



Frusemide

250 mL standard flask. CAS was standardized using ferrous ammonium sulphate and ferroin indicator. XC (0.1%) and safranin O (0.1%) were prepared and diluted appropriately to get the working concentration.

Preparation of frusemide (FRU) solution

Standard drug solution (1000 µg mL^{-1}) was prepared by dissolving 0.1 g of FRU in 50 mL absolute alcohol and diluting to the mark in a 100 mL calibrated flask. The stock solution was diluted appropriately to get the working concentration.

Determination of frusemide

Method A

Aliquots containing 20.00-30.00 µg mL^{-1} of FRU were transferred into a series of 10 mL standard flasks using a micro burette. To this, 1 mL of standardized CAS (0.3%) was added followed by 1 mL of 2 N H_2SO_4. Then 1 mL of 0.05% of XC was added. The contents were shaken well and diluted up to the mark with distilled water. The absorbance of each solution was measured at 612 nm against the corresponding reagent blank.

Method B

Aliquots containing 6.00-16.00 µg mL^{-1} of FRU were transferred into a series of 10 mL standard flasks using a micro burette. To this, 1 mL of standardized CAS (0.3%) was added followed by 1 mL of 2 N H_2SO_4. Then 2 mL of 0.01 % of SAF was added. The contents were shaken well and diluted up to the mark with distilled water. The absorbance of each solution was measured at 526 nm against the corresponding reagent blank.

Assay of formulations

Lasix -40 mg (Aventis Pharma Ltd. India), Amifru -40 mg (Elder Pharmaceuticals Ltd. India) tablets were used in the investigation. Two tablets each of Lasix and Amifru equivalent to 80 mg were powdered and transferred into 2 different 100 mL volumetric flasks by filtration and washing with absolute alcohol. The solution was made up to the mark with distilled water. A convenient aliquot was then subjected to analysis by the proposed method.

Results and Discussion

The method is based on the reaction of FRU with CAS and the unreacted CAS bleaches the coloured dyes to colourless leuco form which shows absorption maximum at 612 nm (Method A) and at 526 nm (Method B). When known excess of CAS was added to an increasing concentration of FRU there was a decrease in the concentration of CAS. When known volume of the dye was added to the same mixture it showed an increase in the concentration of the dye. The result could be observed by increase in the absorbance with the increase in the concentration of FRU at the respective λ_{max}. The reaction mechanisms are represented in Scheme 1. Preliminary experiments were performed to fix the initial concentration of CAS, it was found that 300 µg mL^{-1} of CAS is sufficient to bleach the dye. The reaction was carried out in sulphuric acid medium. One mL of 2 M acid was used in the reaction, as this concentration was found ideal.

Analytical data

A linear correlation was found between absorbance at λ_{max} and concentration ranges given in Table 1. Sensitivity parameters such as molar absorptivity, Sandell's sensitivity, detection limit and quantification limit are 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.

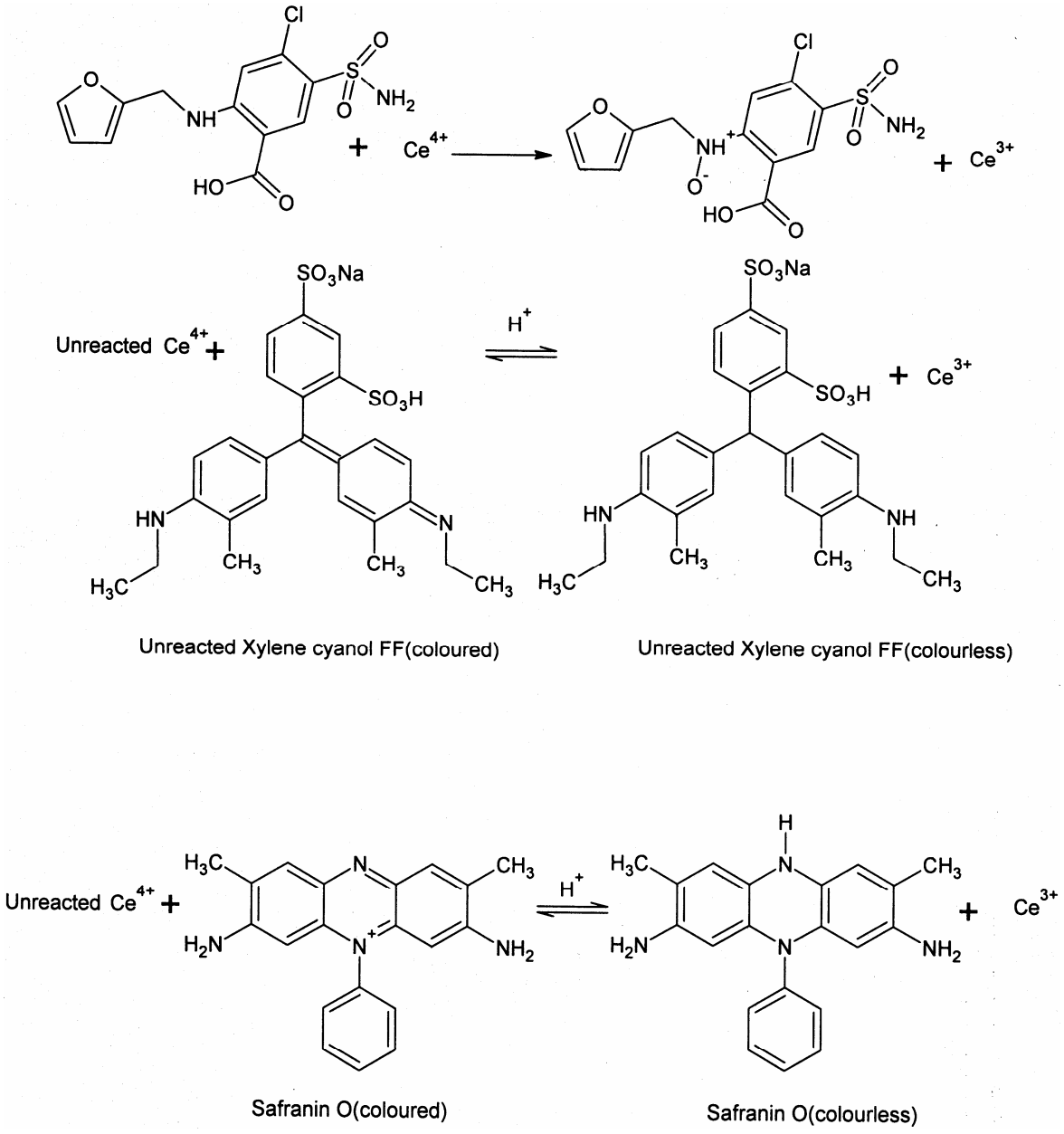
Accuracy and Precision

The accuracy and precision of the methods were established by analyzing the pure drug solution at 5

Table 1—Analytical parameters

	Method A	Method B
λmax (nm)	612	526
Beer's law limit (µgmL^{-1})	20.00-30.00	6.00-16.00
Molar absorptivity ($Lmol^{-1}cm^{-1}$)	1.160×10^{4}	2.025×10^{4}
Sandell's sensitivity (µg cm^{-2})	2.82×10^{-3}	1.63×10^{-2}
Limit of detection** (µgmL^{-1})	0.1612	0.0897
Limit of quantification** (µgmL^{-1})	0.4885	0.2718
Regression equation*	Y=a+bX	Y=a=bX
Slope (b)	0.3664	0.6363
Intercept (a)	-0.0242	-0.0369
Correlation coefficient (r)	0.9992	0.9996

*Y is the absorbance and X is the concentration in (µgmL^{-1})
**calculated using ICH-Guidelines



Scheme 1

different levels (within working limits).The relative error (%) which is a measure of accuracy & RSD (%) a measure of precision are summarized in Table 2 and reveal the high accuracy and precision of the methods.

Interference study

In pharmaceutical analysis, it is important to test the selectivity towards the excipients added to the pharmaceutical preparations. Commonly encountered excipients such as glucose, starch and talc did not interfere in the determination of studied drug.

Application

The proposed method had been successfully applied to the determination of FRU in two different branded tablets. The content of the tablet formulation was calculated by applying suitable dilution factor. The results for two tablets were compared statistically with those of the tabulated value at 95% confidence level. The calculated student's t-test (Table 3) did not exceed the tabulated value, indicating that there was no significant difference between the proposed method and the tabulated value in respect to accuracy and precision.

Table 2—Evaluation of Accuracy and Precision

Method A

Amount taken ($\mu g\ mL^{-1}$)	Amount found* ($\mu g\ mL^{-1}$)	RE (%)	SD ($\mu g\ mL^{-1}$)	RSD (%)
22.00	22.16	0.72	0.022	0.099
24.00	24.08	0.33	0.018	0.074
26.00	26.04	0.15	0.009	0.036
28.00	28.08	0.28	0.018	0.064

Method B

Amount taken ($\mu g\ mL^{-1}$)	Amount found* ($\mu g\ mL^{-1}$)	RE (%)	SD ($\mu g\ mL^{-1}$)	RSD (%)
6.00	5.92	1.33	0.0130	0.7696
8.00	7.94	1.00	0.0110	1.3853
10.00	9.88	1.20	0.0238	2.4089
12.00	12.10	0.83	0.0173	1.4297
14.00	14.02	0.14	0.0057	0.4065
16.00	16.08	0.50	0.0295	1.8345

*Mean value of five determinations
RE-Relative Error, SD-Standard Deviation; RSD-Relative Standard Deviation

Table 3—Result of assay of formulations by the proposed method

Method A

Brand name	Labeled amount (mg)	Amount found* (mg)	% label claim±S D
Lasix[a]	40.00	40.28	100.7±0.02 t=1.63
Amifru[b]	40.00	40.09	100.3±0.02 t=1.0

Method B

Brand name	Labeled amount (mg)	Amount found* (mg)	% label claim±S D
Lasix[a]	40.00	40.16	100.4±0.0167 t=0.53
Amifru[b]	40.00	39.60	99±0.0167 t=1.87

*Mean of five determinations
[a]Aventis Pharma Ltd, [b]Elder Pharmaceutical Ltd.
Tabulated t-value at 95% confidence level is 2.77

Conclusion

Simple and rapid method for the determination of FRU has been developed. The method is easy to perform and do not contain any stringent experimental variables which effect the reliability of the results. There is no interference from common additives and excipients. The method thus can be used in the determination of FRU in pure and dosage forms.

Acknowledgement

The authors gratefully acknowledge the receipt of pure Frusemide sample from Ariane Orgachem Pvt. Ltd., Mumbai.

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