International Journal of Pharmaceutics, 99 (1993) 333–336

IJP 03177

Stability-indicating derivative spectrophotometric determination of frusemide

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(Received 20 August 1992)
(Modified version received 21 December 1992)
(Accepted 23 December 1992)

Key words: Frusemide determination; Degradation product; Derivative spectrophotometry; Kinetics; Dosage form

Summary

The development of a first- and second-derivative spectrophotometric determination of frusemide in pharmaceutical formulations in the presence of its acid-hydrolysis product 'saluamine', is described. The procedure involves measurements of first-derivative amplitudes at 254 and 262 nm ($^1D_{254}$ and $^1D_{262}$) and second-derivative amplitudes at 265 and 272 nm ($^2D_{265}$ and $^2D_{272}$) for the assay of frusemide and saluamine, respectively. Kinetic investigation of the acid degradation rate of frusemide revealed that the proposed method is stability indicating. Tablets and injections have been assayed and the results indicated that the proposed derivative spectrophotometric method is as accurate and precise as the official method.

Frusemide is a potent diuretic widely prescribed for the treatment of oedema and essential hypertension (Martindale, 1989). It is formulated in pharmaceutical preparations mainly as tablets and injections. The latter is available in ampoules which contain sodium frusemide equivalent to 10 mg of frusemide per ml and is prepared with the aid of sodium hydroxide. The product is formulated to a pH between 8 and 9.3 (British Pharmacopoeia, 1988) or between 8.5 and 9.3 (US Pharmacopoeia, 1985) because the furfuryl group is acid labile and frusemide is reported to hydrolyse to saluamine (Cruz et al., 1979). Pharmacopoeial methods for the determination of frusemide in tablets and injections are based on measuring the absorbance at 271 nm (US Pharmacopoeia, 1985; British Pharmacopoeia, 1988). A survey of the literature revealed that there are numerous methods which are concerned with the analysis of frusemide in dosage forms. These methods include titrimetry (Shrirama et al., 1980), spectrophotometry (Moustafa and Abdel-Moety, 1987), colorimetry (Sastry et al., 1988; Issopoulos, 1989), coulometry (Nikolic and Velasevic, 1989) and high-performance liquid chromatography (Roth et al., 1981; Rapaka et al., 1982; Neil et al., 1984; Rao and Raghuveer, 1985; Stoberski et al., 1988). Such methods either require tedious sample manipulation or lack specificity. Therefore,

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the objective of the present work was to develop a stability-indicating method based on the use of derivative spectrophotometry for the direct analysis of frusemide in presence of its degradation product, saluamine.

Solutions of either frusemide or saluamine of 0.2 mg/ml were prepared in methanol and stored refrigerated at 4°C in brown glass flasks.

Analysis was carried out using a Shimadzu UV-240 recording spectrophotometer equipped with an optional program/interface, Model OPI-2, giving from-first to-fourth derivatives. The instrument parameters were: spectral slit width, 2 nm; scan speed, 10 nm/s; recorder chart speed, 10 nm/cm; wavelength range, 230–330 nm; and ordinate maximum and minimum settings, ±0.1 (first derivative) and ±0.04 (second derivative).

The UV absorption (zero-order) spectra of frusemide and its hydrolysis degradation product, saluamine, in 0.1 M NaOH solution are very similar (Fig. 1) and neither spectrum shows prominent peaks that can be used for reliable direct absorbance measurements. First-derivative spectra of frusemide and saluamine at various concentrations (Fig. 2) show that there are distinct iso-differential points at 254 and 262 nm irrespective of the concentrations of saluamine and frusemide, respectively. Similarly, iso-differential points in the second-derivative spectra of saluamine and frusemide were found at 265 and 272 nm, respectively. Therefore, the first-derivative amplitudes at 254 nm (zero-crossing of saluamine) and 262 nm (zero-crossing of frusemide), and the second-derivative amplitudes at 265 nm (zero-crossing of salumine) and 272 nm (zero-crossing of frusemide) were chosen for the simultaneous determination of the two compounds in mixtures.

Linearity was assessed by construction of a six-point calibration graph over the concentration range 0.5–5 μg/ml. The ^{1}D amplitudes at 254 nm and ^{2}D amplitudes at 265 nm for frusemide and ^{1}D amplitudes at 262 nm and ^{2}D amplitudes at 272 nm for saluamine, were measured graphically and plotted against the corresponding concentration. Least-square regression analysis was carried out for the slope, intercept, and correlation coefficient. In all cases, a proportional relation was established, with correlation coefficients ⩾ 0.9997 and relative standard deviation (RSD%) ⩽ 2.10% (Table 1) which indicates good precision and reproducibility of the proposed derivative method.

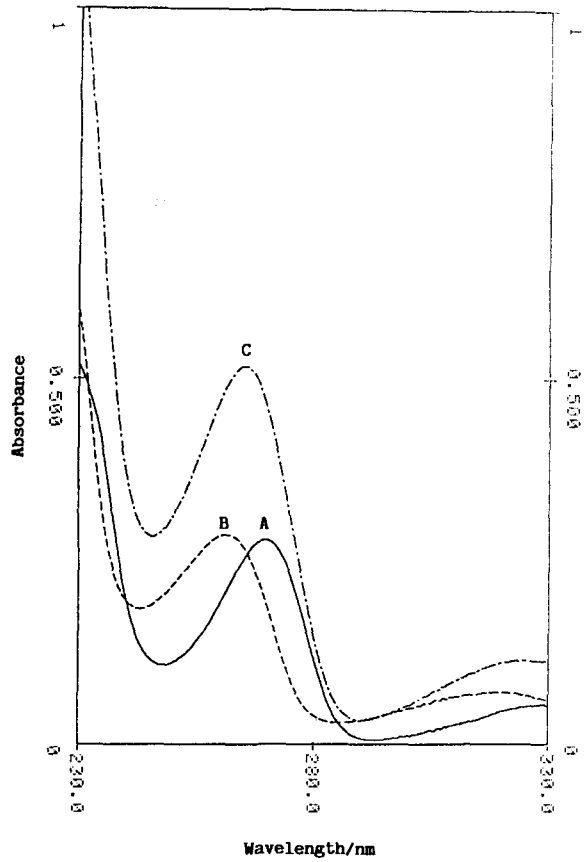

Fig. 1. Zero-order spectra of A (———) frusemide (5 μg/ml), B (- - - - -) saluamine (5 μg/ml) and C (— — —) a mixture of frusemide (5 μg/ml) and saluamine (5 μg/ml) in 0.1 M sodium hydroxide solution.

To assess the method specificity for assay of frusemide without interference from the its degradation product, saluamine, stability investigations were conducted. Solutions of frusemide of 1 μg/μl were prepared in 0.1 M HCl and kept in light-resistant flasks incubated at 80 ± 0.1°C. Samples, of 40 μl, were withdrawn at appropriate intervals and transferred into 10-ml volumetric flasks and made up to volume with 0.1 M sodium

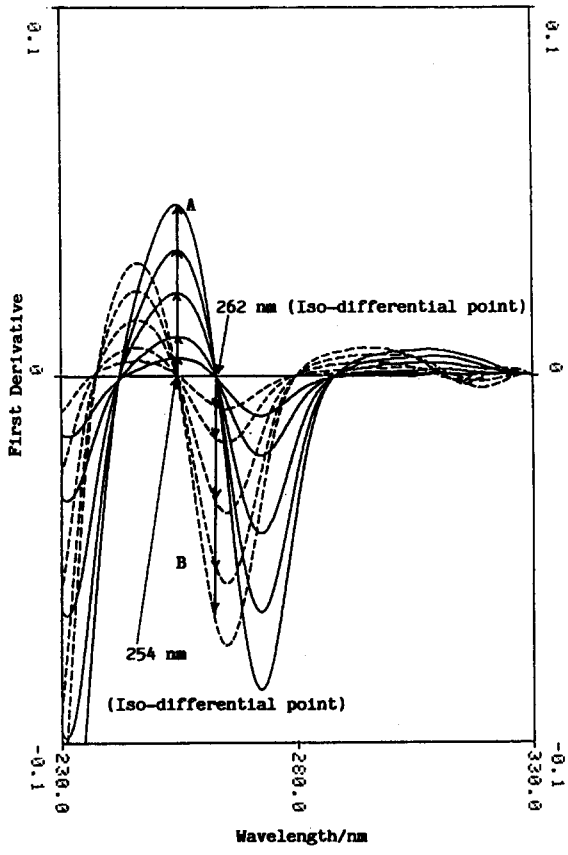

Fig. 2. First-derivative spectra of frusemide (A, ———) and saluamine (B, - - - - - -) at several different concentrations in 0.1 M sodium hydroxide solution: frusemide, 0.5, 1.0, 2.0,3.0 and 4.0 μg/ml; saluamine, 0.5, 1.0, 2.0, 3.0 and 4.0 μg/ml.

hydroxide solutions, then analyzed for frusemide using first- and second-derivative spectrophotometry. A regular decrease in the concentration of the intact drug with increasing time intervals was observed. The $^1D_{254}$ or $^2D_{265}$ values for the residual drug in the samples have been measured, from which the corresponding log (C, %) value was calculated and correlated to the time intervals. Linear plots with regression coefficients in the range 0.9970–0.990 were obtained, indicating that the degradation rate was first order. The slopes of the linear plots, the half-life periods ($t_{1/2}$) and the degradation rate constants (k) are -0.0022, -0.0021; 136 min, 144 min; and 0.0051 min^{-1}, 0.0048 min^{-1} for ^{1}D and ^{2}D, respectively. The results proved that the proposed derivative methods are specific for determining frusemide without interference from its acid-induced degradation product, saluamine.

The applicability of the proposed method has been appraised further through the assay of the dosage forms either as tablets or injections.

An accurately weighed portion of the powdered tablets equivalent to approx. 40 mg of frusemide was transferred to a 100 ml volumetric flask and dispersed in about 70 ml methanol. After sonication for 15 min, the volume was adjusted to 100 ml with methanol and filtered through a Millipore filter. Then, 400 μl of this solution was diluted to 100 ml with 0.1 M sodium hydroxide solution and the derivative absorption spectra were recorded. The contents of one ampoule was diluted to 100 ml with 0.1 M sodium hydroxide solution. 200 μl of this solution was pipetted accurately and diluted to 10 ml with 0.1 M sodium hydroxide solution and the derivative absorption spectra were recorded.

TABLE 1

Analytical data for the calibration curves ($n = 6$) for the determination of frusemide and saluamine by first- and second-derivative UV spectrophotometry

Compound	Method	Linearity range (μg ml^{-1})	Regression equation		Correlation coefficient r	RSD [a] (%)
			Slope	Intercept		
Frusemide	$^1D_{254}$	0.5–5.0	8.585	−0.471	0.9997	1.03
	$^2D_{265}$	0.5–5.0	12.000	−0.445	0.9999	1.11
Saluamine	$^1D_{262}$	0.5–5.0	13.197	−0.542	0.9997	1.58
	$^2D_{272}$	0.5–5.0	7.486	−0.421	0.9999	2.10

[a] Percent relative standard deviation.

TABLE 2

Assay results for the analysis of frusemide in commercial formulations by first- and second-derivative UV spectrophotometry and the official USP method

Method	Tablets [a]		Injections [a]	
	Found (% declared strength)	RSD (%)	Found (% declared strength)	RSD (%)
$^1D_{254}$	97.73	1.00	97.75	0.90
	t [c] = 0.94		t [c] = 0.63	
	F [d] = 0.47		F [d] = 0.66	
$^2D_{265}$	97.41	1.00	98.04	1.17
	t [c] = 1.38		t [c] = 0.92	
	F [d] = 0.80		F [d] = 1.13	
USP [b]	98.39	1.31	97.36	1.12

[a] Mean and relative standard deviation (RSD%) of six determinations given as percentage of the claimed content (40 mg per tablet; 20 mg per ampoule).
[b] US Pharmacopoeia.
[c] Calculated t values for which the theoretical t ($p = 0.05$) is 2.23.
[d] Calculated F values for which the theoretical F (95%) is 5.05.

The results obtained were compared with those of the official USP (US Pharmacopoeia, 1985) method using the t-test for accuracy and the F-test for assessment of precision. The calculated values did not exceed the corresponding theoretical values, indicating that the difference between the results of the proposed and comparative method was insignificant (Table 2).

Being simple, specific, of good accuracy and high precision, the proposed method can be recommended for the routine analysis and quality control of frusemide in dosage forms and is suitable as a rapid alternative to chromatographic methods. In addition, the proposed method takes into consideration the possible presence of the degradation product saluamine, hence it does indicate the stability of frusemide.

Acknowledgment

The author wishes to express his gratitude to Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ, for the gift of pure frusemide and saluamine which made this study possible.

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