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COMMUNICATIONS

Application of difference spectroscopy to the determination of some pharmaceutically important nitro compounds

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Abstract—A simple and selective spectrophotometric method for the determination of some pharmaceutically important nitro compounds has been developed. The suggested method depends upon the spectral changes induced by reduction using either Zn/HCl or Zn/NH_4Cl. The different experimental parameters were studied and incorporated into the procedure. The mean percentage recovery ranged from 99 to 101. The proposed method was applied to the determination of the studied compounds in dosage forms, and the results obtained were compared favourably with those given with the compendial ones.

Organic nitro compounds belong to different pharmacological classes, and this makes the determination of this functional group of broad interest. Analytical methods for the nitro group have concentrated on the reduction to the corresponding amine, or to one of the several intermediate nitrogen oxidation states. We have developed a simple spectrophotometric method based on the difference between the absorbance of the compound and the absorbance of its reduction product. The difference was linearly related to the analyte concentration in the range 0·4–1·2 mg/100 mL. The proposed method is simple, accurate and can be applied to the determination of the nitro compounds in the presence of other compounds having absorption spectra not affected by reduction. Moreover, it may be considered as a general method for organic nitro compounds.

Materials and methods

Apparatus. Spectrophotometer, Pye Unicam SP 1800, equipped with a matched pair of 1 cm quartz cells.

Reagents. Hydrochloric acid, AR grade, ammonium chloride 10% aqueous solution, methanol, zinc powder, dimethyl formamide.

Materials. Furazolidone, nitrofurantoin, nitrofurazone, nifuroxime nitrazepam, niridazole and metronidazole, were kindly provided by various manufacturers and were used as received. Dosage forms containing the compounds were randomly collected from commercial sources.

Sample preparation. A stock solution containing 1·0 mg mL^{-1} in the appropriate solvent (Table 1) is prepared.

Procedure A. Reduction with Zn/NH_4Cl. Transfer 5·0 mL of the stock solution to a 50 mL calibrated flask, add 20 mL of 10%

Table 1. Collective data for the reduction of the nitro compounds using Zn/HCl and Zn/NH_4Cl.

No.	Compound	Solvent	Concentration mg/100 ml	Reduction time (min)	max (nm)	ΔA 1%, 1cm Zn/NH_4Cl	ΔA 1%, 1cm Zn/HCl
1	Furazolidone	DMF	0·4–1·2	10	260	412 (0·83)	469 (1·05)
					365	573 (0·41)	560 (0·54)
2	Nitrofurazone	DMF	0·4–1·2	10	262	598 (1·0)	696 (0·64)
					375	736 (0·69)	798 (0·73)
3	Nitrofurantoin sodium	Water	0·4–1·2	10	270	348·5 (1·1)	414 (0·86)
					376	527 (0·68)	252 (1·05)
4	Nitrazepam	Methanol	0·4–1·2	15	258	209 (1·2)	341 (0·94)
					310	275 (1·0)	351 (0·64)
5	Niridazole	DMF	0·4–1·2	15	368	716 (0·31)	716 (0·31)
6	Metronidazole	Water	0·2–1·0	10	320	739 (0·14)	739 (0·14)
7	Nifuroxime	Methanol	0·2–1·6	20	231	318·73 (1·6)	381·23 (0·206)
					340	676·7 (0·35)	123·28 (1·9)

N.B. 1– The results are the average of 15 separate determinations.
2– The figures in parentheses are the coefficients of variation.

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ammonium chloride solution followed by 0·3 g zinc powder. Leave for the specified time (Table 1) then complete to the mark with ammonium chloride solution. Filter through a dry filter paper. Transfer 5 mL of the filtrate to a 50 mL calibrated flask and complete to the volume with distilled water. Measure the absorbance of the resulting solution at the wavelength of maximum absorbance (Table 1) against a reagent blank.

Procedure B. Reduction with Zn/HCl. Transfer 5·0 mL of the stock solution to a 50 mL calibrated flask, add 10 mL distilled water, 2 mL hydrochloric acid followed by 0·3 g of zinc powder. Leave to stand for the specified time, then complete to the volume with distilled water. Filter through a dry filter paper and transfer 5·0 mL of the filtrate to a 50 mL calibrated flask and complete to the mark with distilled water. Measure the absorbance of the resulting solution at the specified wavelength (Table 1) against a reagent blank.

In both procedures, subtract the absorbance of the reduced solution from the absorbance of the unreduced solution of the same concentration, to get the absorbance difference (ΔA).

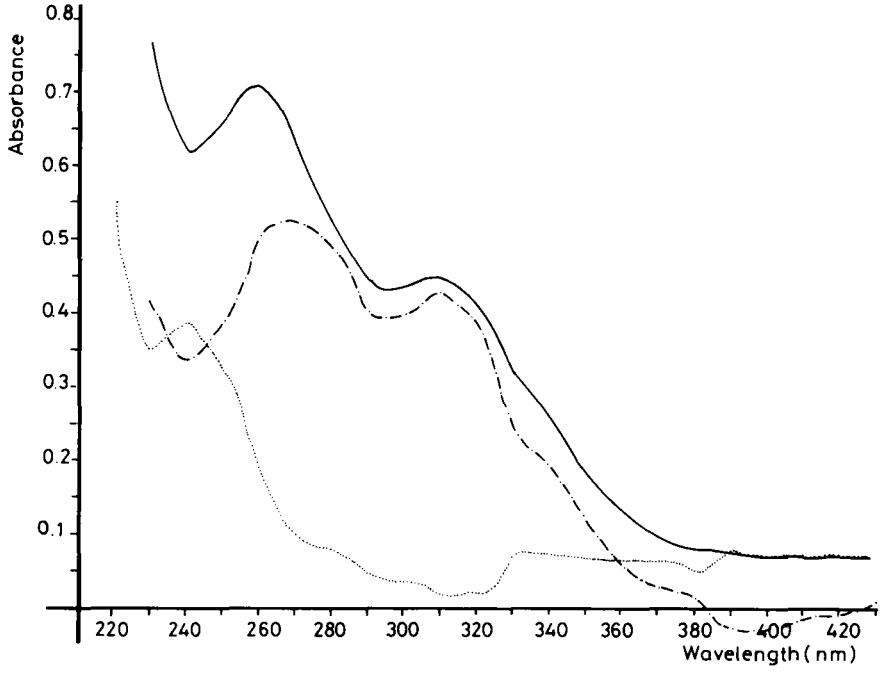

FIG. 1. The absorption spectra of nitrazepam in aqueous methanol (———), its reduction product using Zn/HCl (. . . . . .) at a concentration of 10 μg mL^{-1}, and the delta A absorption curve derived from (– · – · –).

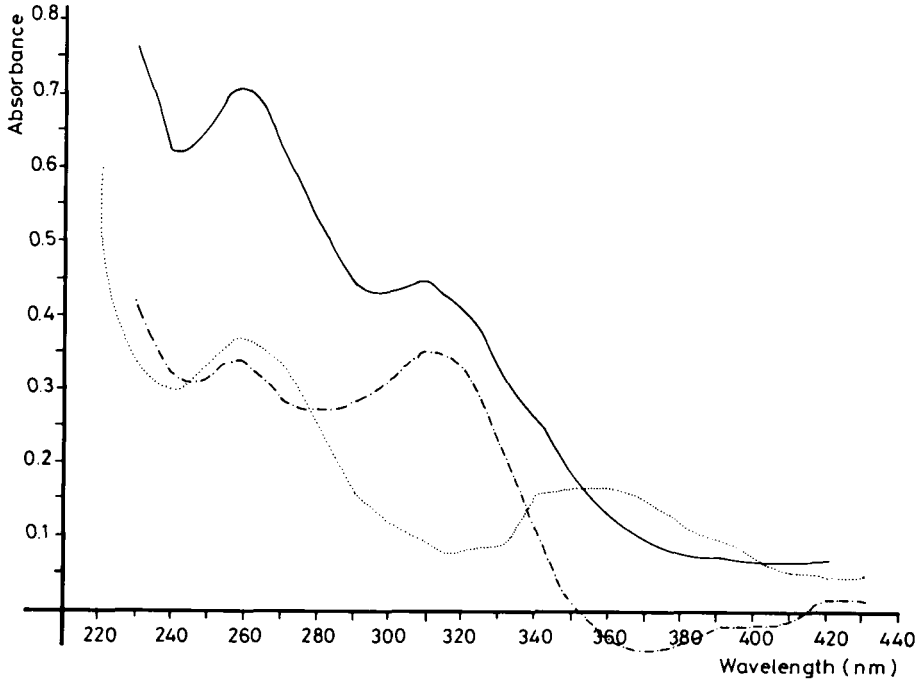

FIG. 2. The absorption spectra of nitrazepam in aqueous, methanol (———), its reduction product using Zn/NH_4Cl (. . . . . .) at a concentration of 10 μg mL^{-1}, and the delta A absorption curve derived from (— · — · —).

Table 2. Statistical comparison between the results of both the proposed and official methods using the Student's *t*- and F-tests.

Compound	Mean recovery* %, (±F.L.) Zn/HCl method		Official method		Zn/NH_4Cl method
Furazolidone	100·1±0·50		99·7±0·27		99·3±0·49
t		(1·15)		(1·15)	
F		(3·42)		(3·36)	
Metronidazole	100·7±0·55		101·1±0·34		99·9±0·65
t		(1·06)		(3·03)	
F		(2·48)		(3·64)	
Nifuroxime	100·2±0·54		100·0±0·56		99·4±0·91
t		(0·48)		(1·41)	
F		(1·10)		(1·06)	
Niridazole	99·6±0·806		101·1±0·69		99·4±1·03
t		(1·03)		(1·34)	
F		(1·37)		(2·25)	
Nitrazepam	99·2±0·41		100·2±0·62		99·7±0·46
t		(1·75)		(1·21)	
F		(2·33)		(1·84)	
Nitrofurantoin	99·7±0·70		99·3±0·93		98·8±0·77
t		(0·79)		(0·96)	
F		(1·77)		(1·45)	
Nitrofurazone	99·7±0·65		99·5±0·68		98·8±0·40
t		(0·42)		(1·64)	
F		(1·35)		(3·61)	

* The mean recovery is the average of 6 separate determination at the longer wavelength.
* Tabulated value of $t = 2{\cdot}228$.
* Tabulated value of $F = 5{\cdot}1$.

Calculate the concentration of the sample, using either a previously plotted calibration graph or from the value of ΔA(1%, 1 cm).

Application to dosage forms

Tablets. Weigh and pulverize 20 tablets. Transfer about 100 mg powder accurately weighed into a beaker, extract with three 25 mL portions of the appropriate solvent (Table 1) and filter into a 100 mL calibrated flask. Wash and transfer the washings to the calibrated flask. Fill to the mark with the same solvent. Transfer 5·0 mL of this solution to a 50 mL calibrated flask and proceed as described under procedure A or B.

Medicated powder for external use. Mix the contents of 5 containers. Transfer an accurately weighed amount of the homogenized powder equivalent to about 100 mg of the drug into a small flask, and proceed as described under 'tablets'.

Procedure for ointment. Mix the contents of 10 tubes. Transfer an accurately weighed amount of the mixed ointment, equivalent to about 100·0 mg of the drug. Melt on a water bath. Extract as described under 'tablets' and proceed as described under procedure A or B.

Results and discussion

Figs 1 and 2 show the absorption spectra of nitrazepam as a model example—before and after reduction and the ΔA absorption curves derived therefrom, using Zn/HCl and Zn/NH_4Cl, respectively. Reduction of nitro compounds using Zn/HCl produces the corresponding primary amine, while Zn/NH_4Cl produces the hydroxylamine derivative (Stenlake 1979). Both the amino derivative and the hydroxylamine derivative show relatively lower absorbance. The absorbance difference, Δ A was found to be linear with the concentration of the analyte in the range cited in Table 1.

Furazolidone, nitrofurantoin, nitrofurazone, nitrazepam and nifuroxime each has two wavelengths suitable for intensity measurement. However, since the intensity at the longer wavelength is greater than that of the shorter wavelength, the former is recommended.

Table 1 summarizes the various experimental conditions and data of the proposed methods. Authentic samples of the pure compounds were analysed by each of the proposed methods and by the official methods (BP 1980 and USP 1985) from aliquots containing the drugs over the concentration range cited in Table 1 and the corresponding concentration was calculated using either the delta A (1%, 1 cm) at the specified λ max or the previously plotted calibration graph.

Table 2 shows the statistical analysis of the results obtained by the proposed methods and the official methods, using the Student's *t*- and the variance ratio F-tests (Hinchen 1966). As evident, there is no significant difference between the two methods as regard to the accuracy and precision. The proposed methods were applied to the determination of preparations (tablets, powders, creams). The results showed agreement with those given with the official methods being within ±1·1%.

In addition to the simplicity and accuracy of the proposed method, it has the advantages that it can be applied to the determination of the nitro compounds in presence of other compounds having absorption spectra not affected upon reduction under the described conditions, and it can be considered as a general method for organic nitro compounds.

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