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A Simple and Sensitive Spectrofluorimetric Method for Analysis of Some Nitrofuran Drugs in Pharmaceutical Preparations

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Abstract A simple, rapid, selective and sensitive spectrofluorimetric method was described for the analysis of three nitrofuran drugs, namely, nifuroxazide (NX), nitrofurantoin (NT) and nitrofurazone (NZ). The method involved the alkaline hydrolysis of the studied drugs by warming with 0.1 M sodium hydroxide solution then dilution with distilled water for NX or 2-propanol for NT and NZ. The formed fluorophores were measured at 465 nm (λ_{Ex} 265 nm), 458 nm (λ_{Ex} 245 nm) and 445 nm (λ_{Ex} 245 nm) for NX, NT and NZ, respectively. The reaction pathway was discussed and the structures of the fluorescent products were proposed. The different experimental parameters were studied and optimized. Regression analysis showed good correlation between fluorescence intensity and concentration over the ranges 0.08–1.00, 0.02–0.24 and 0.004–0.050 μg ml^{-1} for NX, NT and NZ, respectively. The limits of detection of the method were 8.0, 1.9 and 0.3 ng ml^{-1} for NX, NT and NZ, respectively. The proposed method was validated in terms of accuracy, precision and specificity, and it was successfully applied for the assay of the three nitrofurans in their different dosage forms. No interference was observed from common pharmaceutical adjuvants. The results were favorably compared with those obtained by reference spectrophotometric methods.

Keywords Nitrofuran drugs · Nifuroxazide · Nitrofurantoin · Nitrofurazone · Spectrofluorimetric determination · Pharmaceutical preparations

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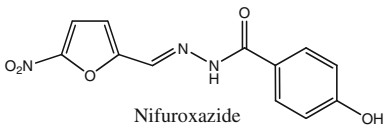
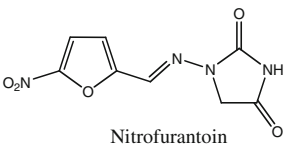
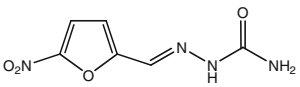
Introduction







Drugs belonging to the group of 5-nitrofuran derivatives are well known and widely used due to their antimicrobial activity. Their mode of action is uncertain but appears to depend on the formation of reactive intermediates by reduction, which inactivate or alter bacterial ribosomal proteins and other macromolecules. Bacteria reduce nitrofuran drugs more rapidly than do mammalian cells, and this is thought to account for the selective antimicrobial activity of these compounds. Nitrofurantoin (NT) is bactericidal to many gram-positive and gram-negative pathogens. It is readily absorbed from the gastrointestinal tract and is used in the treatment of urinary tract infections. Nifuroxazide (NX) is poorly absorbed from the gastrointestinal tract and is extensively used as an intestinal antiseptic in the treatment of colitis and diarrhea. Nitrofurazone (NZ) is bactericidal against most bacteria commonly causing surface infections. It is used as a local application for wounds, burns, ulcers, skin infections and for the preparation of surfaces before skin grafting [1].

NX is an official drug in the British Pharmacopoeia (BP) [2] and it is assayed by potentiometric titration with 0.1 M sodium hydroxide. NT and NZ are official in both the BP [2] and the United States Pharmacopoeia (USP) [3] and

their bulk powders and pharmaceuticals are assayed by either A_{max} spectrophotometric or high-performance liquid chromatography (HPLC) methods.

Several analytical methods were reported in the scientific literature for the determination of the three selected nitrofuran drugs. NX was assayed in its pharmaceutical preparations using direct spectrophotometric methods [4, 5]. Several polarographic and voltammetric techniques were applied for the analysis of NX in pharmaceutical formulations [4–7] and in biological fluids [7, 8]. Also, HPLC [9] was applied for NX determination in biological fluids. Recently, resolution of mixtures containing NX has been carried out using HPLC [10], thin layer chromatography (TLC)–densitometry [10, 11] and several spectrophotometric methods [10, 11]. Several analytical methods were published for the determination of NT and NZ in pharmaceutical preparations and/or biological fluids, including TLC [12], HPLC [12–18], chemometric spectrophotometry [18], derivative spectrophotometry [18, 19], colorimetry [20–22], differential-pulse polarography [23, 24] and several adsorptive and cathodic stripping voltammetric methods [25–27].

Reviewing the literature revealed that, up to the present time, nothing was reported concerning the spectrofluorimetric determination of NX. On the other hand, spectrofluorimetry and chemiluminescence were applied in the analysis of both NT and NZ. Fluorescence assay of NT was carried out by acid hydrolysis of the drug producing 5-nitro-2-furaldehyde which was coupled with *o*-aminothiophenol to give a fluorescent adduct measured at 422 nm (λ_{Ex} 375 nm) [28]. The same procedure was applied for NZ but concentrated hydrochloric acid was needed for hydrolysis, and heating of the hydrolysis product with the reagent lasted for 1 h prior to fluorescence measurement [29]. Flow injection chemiluminescence was adopted where the measured signals were generated by reaction of the photolysis product of NT with luminal–Co(II) mixture [30] while NZ reacted with *N*-bromosuccinimide–hydrogen peroxide mixture [31]. Fluorescence sensors were utilized for the assay of both NT and NZ based on fluorescence quenching. A naphthalimide derivative fluorescent sensor [32] was applied for the determination of NT tablets and urine samples, while a coumarin derivative was used as fluorescent carrier for the optical chemosensing of NZ [33]. Although the above-mentioned fluorescence-related methods are sensitive however, their lengthy procedures or the requirement of elaborated instruments preclude their use in routine analysis.

Solutions of alkali hydroxides react instantaneously with nitrofuran compounds to give intense colors. This simple reaction has been used as an identification test for NT and NZ [2, 34]. The formed colors fade upon heating resulting in almost colorless solutions showing strong fluorescence which can be the basis for a simple, rapid, selective and sensitive spectrofluorimetric procedure for the analysis of the selected nitrofuran drugs. The proposed method is less expensive and does not need sophisticated instruments. The method has been satisfactorily applied to the analysis of the three nitrofuran drugs in their different commercial products.

Experimental

Apparatus

Fluorescence measurements were carried out using a Shimadzu (Kyoto, Japan) RF-1501 version 3.0 spectrofluorophotometer equipped with a 150 W xenon lamp and 1-cm quartz cells. A thermostated water bath accurate to ±0.5 °C (Köttermann Hänigsen, Germany), was used.

Materials

NX was kindly supplied by Amriya for Pharmaceutical Industries, Alexandria, Egypt. NT and NZ were kindly provided by Medical Union Pharmaceuticals, Abu-Sultan, Ismailia, Egypt. dimethylformamide (DMF) and 2-propanol (BDH Laboratory Supplies, Poole, England) were of analytical-reagent grade. Aqueous solutions were prepared using high purity distilled water.

Pharmaceutical preparations of the three drugs were obtained from the local market. Pharmaceutical preparations of NX include Antinal® capsules (Amoun Pharmaceutical Co., El-Obour City, Cairo, Egypt, BN. 3796), Ercéfuryl® capsules (Amriya for Pharmaceutical Industries, Alexandria, Egypt under license of Laboratoires Synthélabo, Le Plessis Robinson, France, BN. 930501) both labeled to contain 200 mg NX per capsule, Antinal® suspension (Amoun Pharmaceutical Co., El-Obour City, Cairo, Egypt, BN. 1983) labeled to contain 220 mg NX per 5 ml, Ercéfuryl® suspension (Amriya for Pharmaceutical Industries, Alexandria, Egypt under license of Laboratoires Synthélabo, Le Plessis Robinson, France, BN. 951908) labeled to contain 4% *w*/*w* NX and Drotazide® capsules (Eva Pharma for Pharmaceuticals and medical appliances, Egypt) labeled to contain 200 mg NX and 40 mg Drotaverine HCl per capsule.

Pharmaceutical preparations of NT include Macrofuran® capsules (Kahira Pharmaceutical & Chemical Co., Cairo, Egypt, BN. 0520063) and Uvamin® retard capsules (Medical Union Pharmaceuticals, Abu-Sultan, Ismailia, Egypt under license of Mepha, Basle, Switzerland, BN.

051756) both labeled to contain 100 mg NT macrocrystals per capsule.

Pharmaceutical preparations of NZ include Furaseen® soluble dressing (Chemipharm Pharmaceutical Industries, 6th October City, Egypt, BN. 060235) and Topifuran® ointment (Medical Union Pharmaceuticals, Abu-Sultan, Ismailia, Egypt, BN. 061451) both labeled to contain 200 mg NZ per 100 gm.

Solutions and reagent

NX stock solution (500 μg ml^{-1}), NT stock solution (200 μg ml^{-1}) and NZ stock solution (100 μg ml^{-1}), were prepared in DMF. The solutions were found to be stable for at least 1 week if kept in a refrigerator protected from light. Aqueous 0.1 M sodium hydroxide solution was prepared and used in the study.

General procedure

Reaction mixtures were prepared by suitable dilution of the stock solutions with 0.1 M sodium hydroxide solution to reach concentrations 20.0, 4.0 and 1.0 μg ml^{-1} for NX, NT and NZ, respectively. These solutions were placed in a thermostatically controlled water bath at 60±1 °C for 20 min (for NX and NT) and at 50±1 °C for 20 min (for NZ) then they were cooled under tap water.

Solutions for the calibration graphs were prepared by dilution of accurate volumes of the reaction mixtures to reach the concentration ranges mentioned in Table 1. Dilution was made using distilled water for NX solutions and 2-propanol for NT and NZ solutions. Blank solutions were similarly prepared but the drugs were omitted. The fluorescence intensity was measured at the specified excitation and emission wavelengths (Table 1).

Assay of pharmaceutical preparations

For NX and NT capsules

For each preparation, the contents of ten capsules were accurately weighed and the average weight per capsule was determined. An accurately weighed portion of the finely powdered sample equivalent to 50.0 mg for NX or 20.0 mg for NT was extracted into DMF with the aid of shaking and filtered. Dilution of the filtrate was made with DMF in order to obtain final concentrations 500 μg ml^{-1} for NX or 200 μg ml^{-1} for NT. Aliquots were treated with 0.1 M sodium hydroxide solution to prepare the reaction mixtures and further steps described under general procedure were followed.

For NX suspensions

An accurate volume of Antinal® suspension (1.0 ml) or an accurate weight of Ercéfuryl® suspension (1.25 gm) was transferred into a 100-ml volumetric flask, dissolved in DMF with the aid of shaking and diluted to volume with DMF to obtain final concentrations 440 and 500 μg ml^{-1} NX for Antinal® and Ercéfuryl® suspensions, respectively. Aliquots were treated with 0.1 M sodium hydroxide solution to prepare the reaction mixtures as described under general procedure.

For NZ topical preparations

For each preparation, a portion equivalent to 10.0 mg NZ was weighed, transferred into a 100-ml volumetric flask, dissolved in DMF with the aid of shaking and diluted to volume with DMF to obtain final concentration 100 μg ml^{-1} NZ. Aliquots were directly treated with 0.1 M sodium

Table 1 Experimental and analytical parameters for the spectrofluorimetric determination of NX, NT and NZ

Parameter	NX	NT	NZ
Temp. and time	60 °C/20 min	60 °C/20 min	50 °C/20 min
Solvent	Distilled water	2-Propanol	2-Propanol
$\lambda_{Ex}/\lambda_{Em}$ (nm)	265/465	245/458	245/445
Concentration range (μg ml^{-1})	0.08–1.00	0.02–0.24	0.004–0.050
Regression equation F= a + bC	F=4.20+773C	F=6.07+3197C	F=6.90+19070C
Correlation coefficient (r)	0.99986	0.99991	0.99986
S_a	5.008	3.328	6.028
S_b	7.550	21.361	181.751
RSD% of the slope	0.977	0.668	0.953
$S_{y/x}$	4.775	3.574	5.748
LOD (μg ml^{-1})	0.008	0.0019	0.0003
LOQ (μg ml^{-1})	0.027	0.0063	0.0010

hydroxide solution to prepare the reaction mixtures as described under general procedure.

Results and discussion

Spectral characteristics

A preliminary investigation of the native fluorescence characteristics of NX, NT and NZ showed that the three drugs are non-fluorescent either in aqueous acidic medium or in different organic solvents. However, in alkaline medium (0.1 M sodium hydroxide), the three nitrofuran drugs are hydrolyzed into strongly fluorescent hydrolysis products with excitation and emission wavelengths ranging from 245 to 265 nm and from 445 to 465 nm, respectively (Figs. 1, 2 and 3 and Table 1).

Effect of experimental variables

The reaction conditions with respect to the heating temperature and time, the sodium hydroxide concentration and the diluting solvent were optimized to achieve maximum sensitivity. In order to examine the effect of temperature and heating time on the fluorescence of the hydrolysis product, the reaction was carried out at different temperatures (room temp.≈25, 40, 50, 60, 70 and 80 °C) using a thermostatically controlled water bath for periods ranging from 10 to 60 min. At room temperature, the hydrolysis was slow, while increasing the temperature produced an increase in hydrolysis rate and consequently in fluorescence up to 60 °C (for NX and NT) and to 50 °C (for NZ), after which a decrease in fluorescence intensity was observed (Fig. 4). The appropriate temperature and heating time for each drug are given in Table 1.

The influence of concentration of sodium hydroxide was studied and 0.1 M solution was found to be optimum. For NT and NZ, increasing the strength of the alkali solution produced a slight increase in the background fluorescence and a slight decrease in the fluorescence of the hydrolysis product. The situation was much worse for NX because increasing the sodium hydroxide concentration to 0.2 M led to 20% reduction in fluorescence intensity and it further decreased with increasing the alkali solution concentration.

Different diluting solvents were tested. Solvents investigated include water, acids (sulphuric and phosphoric acids), alcohols (methanol, ethanol and 2-propanol), dioxane, DMF and acetone. Acids were not suitable probably due to decomposition of the hydrolysis products in acidic medium. Organic solvents such as dioxane, DMF and acetone were excluded as well due to high background fluorescence and weak fluorescence signals of the analytes. The best response regarding sensitivity, stability of fluorescence readings and low background fluorescence was obtained by using water (for NX) and 2-propanol (for NT and NZ).

The determination of the nitrofuran drugs under the experimental conditions mentioned above, in which the sodium hydroxide concentration was several thousands times the concentration of either of the three drugs, would result in a pseudo-zero-order reaction with respect to the alkali concentration and the rate of reaction (i.e., the rate of formation of the fluorescent product) will be directly proportional to the concentration of the drug under analysis according to the equation:

$$\text{Rate} = \Delta F/\Delta t = K'[C]^n \quad (1)$$

where F is the relative fluorescence intensity, t is the time in seconds, K' is the pseudo-order rate constant, $[C]$ is the

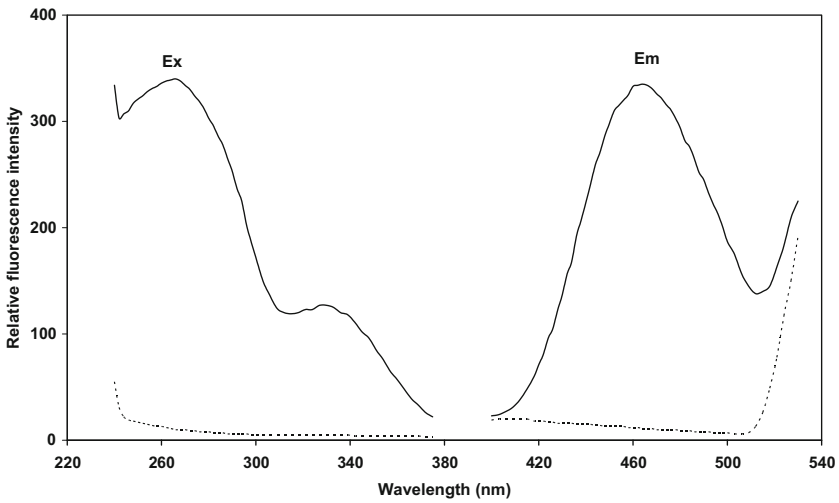


Fig. 1 Excitation and emission spectra of the hydrolysis product of 0.40 μg ml^{-1} NX (—) and the reagent blank (…)

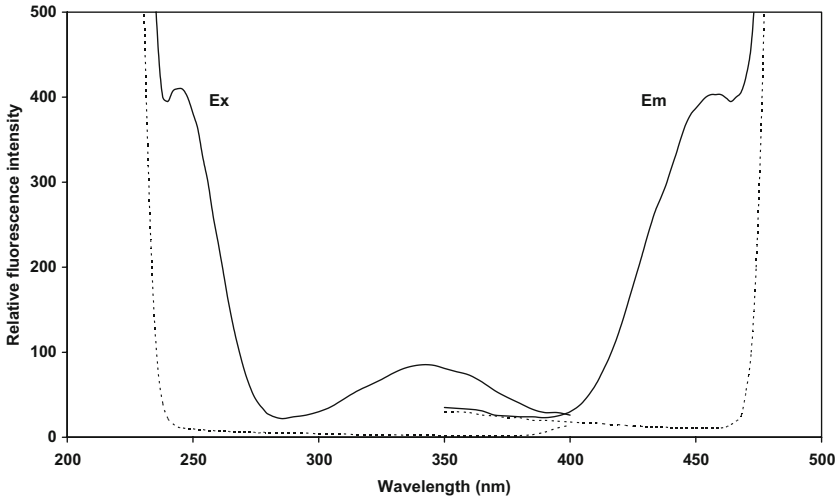


Fig. 2 Excitation and emission spectra of the hydrolysis product of 0.12 μg ml^{-1} NT (—) and the reagent blank (…)

drug molar concentration and n is the order of reaction. The logarithmic form of equation (1) will be:

$$\log(\text{rate}) = \log \Delta F/\Delta t = \log K' + n \log[C] \quad (2)$$

Regression of log $\Delta F/\Delta t$ versus log [C] for each drug revealed that the value of n ranges from 0.968 to 1.002, therefore the reaction follows pseudo-first-order kinetics ($n \approx 1$) with respect to the nitrofurans and log K'=5.262, 5.610 and 6.395 for NX, NT and NZ, respectively.

Stability

The stability of the reaction mixtures (in sodium hydroxide solution) was followed by dilution of aliquots with the selected solvent for each compound at 15 min intervals and measuring their fluorescence intensities. Also, the final measured solutions were tested for their stability. Fluorescence intensity values were stable for at least 2 h.

The reaction pathway

It was reported that alkali hydroxide solutions saponify aliphatic nitro and aromatic polynitro compounds with the liberation of nitrite ions [35]. This reaction proceeds according to the following equation:

$$\text{Ar} - \text{NO}_2 + \text{OH}^- \rightarrow \text{Ar} - \text{OH} + \text{NO}_2^-$$

Some 5-nitroimidazole compounds—which are structurally related to the 5-nitrofurans—were determined colorimetrically using the same principle [36, 37]. The nitrite ions formed by their alkaline hydrolysis were diazotized in

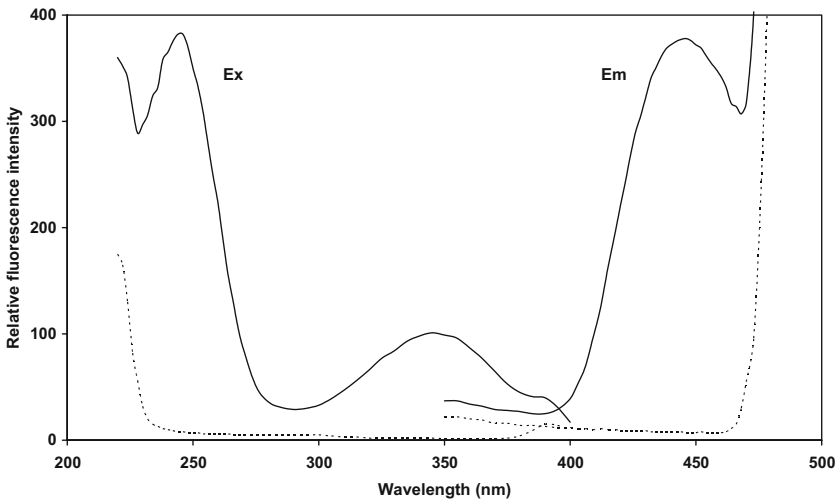


Fig. 3 Excitation and emission spectra of the hydrolysis product of 0.02 μg ml^{-1} NZ (—) and the reagent blank (…)

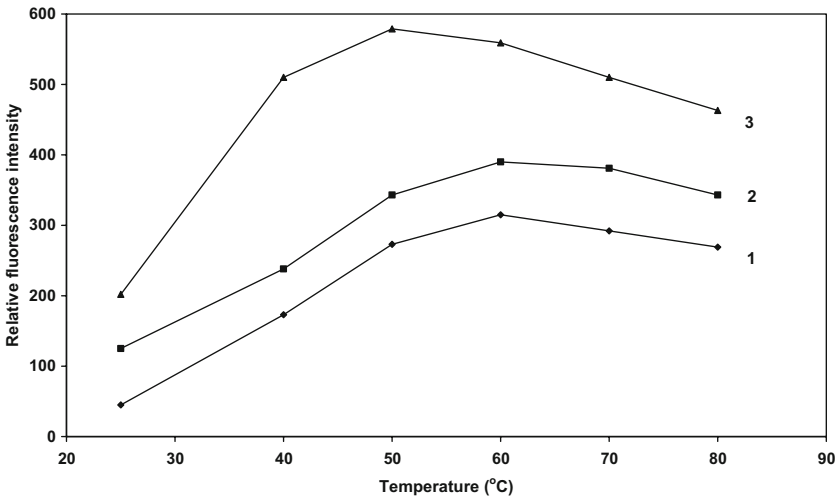
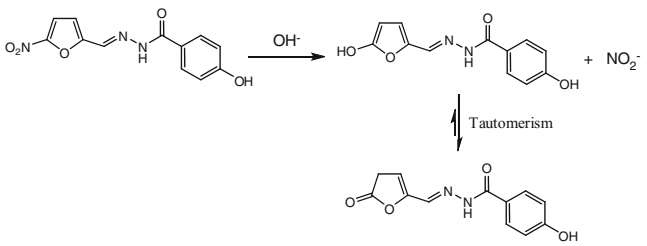


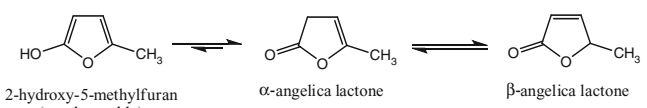
Fig. 4 Effect of temperature on the fluorescence of the hydrolysis products of *1* 0.40 μg ml^{-1} NX, *2* 0.12 μg ml^{-1} NT and *3* 0.03 μg ml^{-1} NZ

acidic medium with sulfanilamide then coupled with *N*-(1-naphthyl)-ethylenediamine dihydrochloride (Bratton–Marshall reagent) to get intense color products.

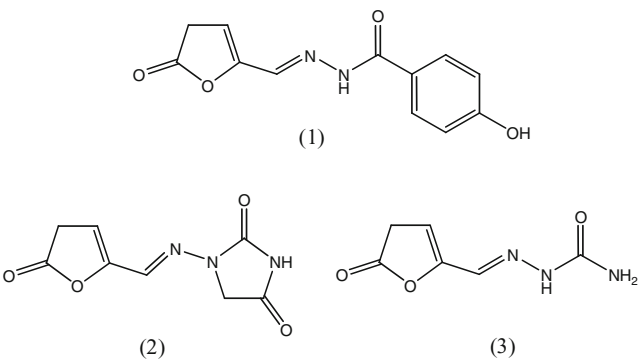
Trukhacheva et al [38] studied the alkaline hydrolysis of NX spectrophotometrically and polarographically. It was found that the interaction of NX with alkali leads to the detachment of the nitro groups in the form of nitrite ions which can be detected spectrophotometrically using the Griess reaction. Also, the polarographic investigation of the reaction showed that the height of the wave corresponding to the reduction of the nitro group in NX linearly decreases with time and this was another evidence for the elimination of the nitro group. Trukhacheva et al [38] explained the alkaline hydrolysis of NX by the substitution of the 5-nitro group to form the 5-hydroxyfuran derivative which undergoes an enol–keto transformation (Tautomerism) to give a lactone structure (butenolide derivative) as shown in the following scheme.



Another piece of evidence for the possible enol–keto transformation of the 5-hydroxyfuran product of NX alkaline hydrolysis is the very similar tautomeric transformation of 2-hydroxy-5-methylfuran into angelica lactones [39].



Based on the above facts, I suggest that the structures 1, 2 and 3 given below are the structures of the fluorescent lactone products of the alkaline hydrolysis of NX, NT and NZ, respectively.



Analytical performance of the method

Concentration ranges and calibration graphs

Under the specified reaction conditions (Table 1), the relative fluorescence intensities measured at the specified working wavelengths were found to be proportional to the concentrations of the three investigated nitrofurans. Table 1 presents the performance data and statistical parameters for the proposed method including linear regression equations, concentration ranges, correlation coefficients, standard deviations of the intercept (S_a), the slope (S_b) and the standard deviation of residuals ($S_{y/x}$). The high values of the correlation coefficients indicate good linearity over the working concentration ranges.

Detection and quantification limits

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated in accordance to the equations

provided by the official compendial methods [3]. The LOD and LOQ are defined as 3 and 10 sb^{-1}, respectively where *s* is the standard deviation of replicate blank responses at the working wavelengths and *b* is the slope of the calibration graph. Both LOD and LOQ values (Table 1) confirms the sensitivity of the proposed spectrofluorimetric method. The high sensitivity of the method allows the detection of concentrations 8.0, 1.9 and 0.3 ng ml^{-1} for NX, NT and NZ, respectively.

Precision and accuracy

The precision and accuracy of the proposed spectrofluorimetric method were examined at three concentrations levels for each drug by five replicate analyses for each concentration. The percentage relative standard deviation (RSD%) and the percentage relative error (E_r%)did not exceed 1.5% proving the high repeatability and accuracy of the method (Table 2). This good level of precision and accuracy is suitable for the quality control analysis of the studied drugs.

Specificity and interference study

In order to judge the specificity of the proposed method, a large number of pharmaceutical compounds co-formulated with the studied nitrofurans in different multi-ingredient preparations [1] were tested. These drugs include drotaverine HCl, neomycin sulphate, phenazopyridine HCl, sulfamethoxazole, sulfadiazine, chlorpheniramine maleate, naphazoline HCl, ephedrine HCl, hydrocortisone, prednisolone, clotrimazole, lidocaine HCl, metronidazole, secnidazole and tinidazole. Application of the proposed procedure to these drugs revealed that none of them interfere in the assay even though some drugs exhibited emission peaks when their solutions were excited at 245 or 265 nm, but these peaks were in the range 320–370 nm away from the working emission wavelengths. This clearly demonstrates the advantage of the wide Stokes shifts (the gap between wavelengths of maximum excitation and emission) for the nitrofurans' fluorophores, which enables their selective measurement without any interference from other fluorescent species.

Trimethoprim was found to show some interference in the assay if it was present in the final measured solution in concentration higher than 0.2 $\mu g ml^{-1}$. As the recommended procedure allows the quantification of NT over the range 0.02–0.24 $\mu g\ ml^{-1}$, therefore NT can be adequately determined if it is present as the major component or in 1:1 mixture with trimethoprim. Pyridoxine HCl (vitamin B_6) also caused some interference when it was present in a final concentration >0.3 $\mu g\ ml^{-1}$.

Excipients and diluents such as starch, lactose, talc, magnesium stearate, glycerin, sucrose, sorbitol and citric acid which are commonly formulated in tablets, capsules and syrup dosage forms, obviously did not interfere with the proposed method. Methyl and propyl parabens which are extensively used as preservatives in pharmaceuticals, were tested and they gave emission peaks at 335 nm which did not interfere with the emission bands of the studied nitrofurans.

Based on all the above findings, the proposed spectrofluorimetric method can be considered highly selective for the analysis of the three nitrofurans.

Analysis of pharmaceutical dosage forms

All the pharmaceutical preparations available in the local market for the three drugs were analyzed using the developed spectrofluorimetric method. The studied drugs could be directly determined without any interference from the capsule additives, suspension components (sugar, flavor, preservative, suspending agent, etc.) or the polyethylene glycols soluble dressing or ointment base. Also, the co-formulated drug, drotaverine hydrochloride, did not interfere in the estimation of NX in Drotazide® capsules. The results obtained including recovery data, RSD% and E_r% values were satisfactory (Table 3).

Reference spectrophotometric methods [4, 5, 10, 22] were adopted for the assay of the three drugs in their commercial products. In the Student's *t*- and the variance ratio *F*-tests, the calculated values did not exceed the theoretical ones at the 95% confidence level which indicated that there were no significant differences between the proposed and the reference methods. It is evident from these results that the proposed method is applicable to the analysis of the three nitrofurans in their dosage forms with comparable accuracy and precision.

Table 2 Precision and accuracy for the determination of NX, NT and NZ using the proposed spectrofluorimetric method

Compound	Nominal value ($\mu g\ ml^{-1}$)	Found ± SD[a] ($\mu g\ ml^{-1}$)	RSD (%)[b]	E_r(%)[c]
NX	0.10	0.1004±0.0015	1.49	0.40
	0.50	0.5940±0.0070	1.18	−1.00
	1.00	0.9921±0.0091	0.92	−0.79
NT	0.04	0.0398±0.00041	1.03	−0.50
	0.12	0.1208±0.00084	0.70	0.67
	0.20	0.1989±0.00170	0.86	−0.55
NZ	0.01	0.00993±0.00009	0.91	−0.70
	0.03	0.02972±0.00028	0.94	−0.93
	0.05	0.04986±0.00031	0.62	−0.28

[a] Mean ± standard deviation for five determinations
[b] % Relative standard deviation
[c] % Relative error

Table 3 Application of the proposed spectrofluorimetric method for the determination of NX, NT and NZ in their pharmaceutical preparations

Preparation name		Spectrofluorimetric method	Reference method
Antinal® capsules	%Recovery ± SD[a]	98.44±1.04	99.07±0.94[d]
	RSD%[b]	1.06	0.95
	E_r%[c]	−1.56	−0.93
		t=1.01, F=1.21	
Ercéfuryl® capsules	%Recovery ± SD[a]	99.24±1.11	98.72±0.56[d]
	RSD%[b]	1.12	0.57
	E_r%[c]	−0.76	−1.28
		t=0.94, F=3.88	
Antinal® suspension	%Recovery ± SD[a]	99.51±1.20	100.28±0.86[e]
	RSD%[b]	1.21	0.86
	E_r%[c]	−0.49	0.28
		t=1.17, F=1.93	
Ercéfuryl® suspension	%Recovery ± SD[a]	99.09±1.28	100.48±1.00[e]
	RSD%[b]	1.29	1.00
	E_r%[c]	−0.91	0.48
		t=1.93, F=1.65	
Drotazide® capsules	%Recovery ± SD[a]	100.62±1.13	101.42±1.36[f]
	RSD%[b]	1.12	1.34
	E_r%[c]	0.62	1.42
		t=1.02, F=1.46	
Macrofuran® capsules	%Recovery ± SD[a]	100.28±1.45	100.58±0.69[g]
	RSD%[b]	1.45	0.69
	E_r%[c]	0.28	0.58
		t=0.43, F=4.39	
Uvamin® retard capsules	%Recovery ± SD[a]	99.73±0.78	99.32±1.17[g]
	RSD%[b]	0.78	1.18
	E_r%[c]	−0.27	−0.68
		t=0.65, F=2.27	
Furaseen® soluble dressing	%Recovery ± SD[a]	100.71±1.05	101.72±0.85[g]
	RSD%[b]	1.04	0.84
	E_r%[c]	0.71	1.72
		t=1.68, F=1.51	
Topifuran® ointment	%Recovery ± SD[a]	101.42±0.99	100.60±1.22[g]
	RSD%[b]	0.98	1.21
	E_r%[c]	1.42	0.60
		t=1.17, F=1.54	

Theoretical values for t and F at P=0.05 are 2.31 and 6.39, respectively

[a] Mean % recovery ± standard deviation for five determinations

[b] Relative standard deviation percentage

[c] Relative error percentage

[d] [4]

[e] [5]

[f] [10]

[g] [22]

Conclusion

The alkaline hydrolysis products formed under the above-mentioned conditions and measured spectrofluorimetrically can be regarded as simple, rapid and sensitive procedure for the determination of the three nitrofurans in bulk form and in pharmaceutical formulations. Considering the limits of detection and/or concentrations ranges, the proposed method is more sensitive than many previously published methods including spectrophotometric [4, 5, 10, 11, 18–22], HPLC [10, 12–16, 18] electro-analytical [4, 5, 23, 24, 27] and chemiluminescence and fluorescence-related methods [28–33]. Another advantage of the proposed method is its high level of selectivity which allows the quantification of the studied nitrofurans without interference of other co-formulated drugs or commonly encountered excipients and additives. In addition, the developed method is accurate, precise and economic. These advantages encourage the application of the developed method in routine quality control analysis of the three nitrofurans.

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