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Sensitive spectrophotometric methods for the determination of amoxycillin, ciprofloxacin and piroxicam in pure and pharmaceutical formulations

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Abstract

Two simple, sensitive and accurate spectrophotometric methods have been proposed for the determination of amoxycillin (AMX), ciprofloxacin (CPF) and piroxicam (PIR) in pure and pharmaceutical preparations. The methods are based on the measurement of absorbances of tris(*o*-phenanthroline) iron(II) [method A] and tris (bipyridyl) iron(II) [method B] complexes at 510 and at 522 nm, respectively. Reaction conditions have been optimized to obtain coloured complexes of higher sensitivity and longer stability. The absorbances were found to increase linearly with increase in concentrations of AMX, CPF and PIR which were corroborated by correlation coefficient values. The complexes obeyed Beer's law over the concentration range of 0.06–5.2, 0.04–7.2 and 0.2–6.5 μg ml^{-1} for AMX, CPF and PIR, respectively, in method A, and of 0.05–8.5, 0.05–9.0 and 0.05–6.5 μg ml^{-1} for AMX, CPF and PIR, respectively, in method B. The developed methods have been successfully applied for the determination of AMX, CPF and PIR in bulk drugs and pharmaceutical formulations. The common excipients and additives did not interfere in their determinations. The results obtained by the proposed methods have been statistically compared by means of Student *t*-test and by the variance ratio *F*-test.

Keywords: Spectrophotometry; Amoxycillin; Ciprofloxacin; Piroxicam

1. Introduction

Amoxycillin (AMX), chemically known as [6*R*-6-(α-*p*-hydroxyphenyl-D-glycylamino) penicillanic acid] trihydrate, is the only phenolic penicillin used as an antibacterial drug. Ciprofloxacin (CPF), 1-cyclopropyl-6-fluoro-4-dihydro-1,4-oxo-7-piperazine-1-ylquinoline-3-carboxylic acid, is an antibacterial agent with a broad spectrum of activity against a variety of gram positive and gram negative bacteria. Piroxicam (PIR), 4-hydroxy-2-methyl-*N*-2-pyridinyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide, is a non-steroidal anti-inflammatory agent used for the treatment of rheumatic diseases. AMX [1–3] and CPF [4] are official in BP and USP while PIR is official in USP [5]. The official methods involve potentiometric titration [1] using mercuric nitrate as titrant in acetate buffer and HPLC methods [2,3]

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for the assay of AMX while those for CPF and PIR involve HPLC methods using phosphoric acid and acetonitrile (87:13), and phosphate buffer and methanol (55:45) as mobile phases, respectively.

Many spectrophotometric methods [6–34] have been reported for the determination of AMX [6–17], CPF [18–27] and PIR [28–34]. But none of these methods are satisfactory for routine quality assurance for one or the other reason. Some of these methods have low sensitivity [6,8–11,13,16,17,20–24,26,27,29–34] or work out only at higher concentrations of the drugs [6,10–12,18,19,26,27,29,31,32] or have less stability [13,14,17,21,22,26,32,34] or involve extraction [9,15,24,25,28,29]. Hence it was felt necessary to develop simple and sensitive spectrophotometric methods which do not suffer from the above limitations for the determination of AMX, CPF and PIR in bulk samples and pharmaceutical preparations using reagents such as Fe(III)-1,10-phenanthroline (FPL) and Fe(III)-2,2′-bipyridyl (FBL). The proposed methods are more advantageous compared to official methods, which are laborious.

2. Experimental

2.1. Reagents

All chemicals used were of either analytical or pharmaceutical grade and quartz processed high-purity water was used throughout. Pure AMX was obtained from Cadila Healthcare Ltd., India and CPF and PIR were obtained from Cipla Ltd., India.

Various pharmaceutical formulations of AMX, CPF and PIR were obtained commercially. These formulations contain only one drug and do not exist in combination with other drugs.

2.2. Solutions

Aqueous solutions of standard AMX, CPF and PIR (1000 μg ml^{-1}) were prepared and stored in amber coloured bottles in a refrigerator. The solutions were diluted as and when required.

FPL and FBL were prepared [35] as follows:

1. FPL was prepared by mixing 0.198 g of 1,10-phenanthroline (PNL) with 2 ml of 1 M HCl and 0.16 g of ferric ammonium sulphate dodecahydrate (FAS) and diluting with distilled water to 100 ml.
2. FBL was prepared by mixing 0.16 g of 2,2′-bipyridyl in 2 ml of 1 M HCl with 0.16 g of ferric ammonium sulphate dodecahydrate (FAS) and diluting with distilled water to 100 ml.

2.3. Apparatus

All absorbance measurements were made on a Hitachi UV–visible spectrophotometer model U-2001 with 1 cm matched quartz cells.

2.4. Assay procedure

Aliquots of standard drug solutions of AMX, CPF and PIR were transferred separately into a series of 10 ml calibrated flasks. To these were added 4, 2 and 8 ml of FPL for AMX, CPF and PIR, respectively in method A and 1 ml of FBL for AMX and PIR, and 3 ml of FBL for CPF in method B. The solutions were heated on a water bath at 80 °C (20 min for AMX, 15 min for CPF), at 70 °C (5 min for PIR in method B) or allowed to stand at room temperature (25 min for PIR in method A). The solutions were cooled, diluted up to the mark with distilled water and mixed well. The absorbances of complexes were measured at 510 nm and at 522 nm in method A and method B, respectively, against corresponding reagent blank. Calibration graphs were plotted.

2.5. Application of the proposed methods

2.5.1. Assay procedure for tablets and capsules

Twenty tablets of the selected drugs were finely powdered or mixed contents of ten capsules were taken. An amount equivalent to 25 mg of the drug was weighed accurately and transferred into a 100 ml beaker. Using a mechanical stirrer the powder was completely disintegrated in distilled water for AMX and CPF and in methanol for PIR. The solution was filtered and the filtrate was

made up to 100 ml with the corresponding solvent. An aliquot of the drug solution was analysed as described earlier.

3. Results and discussion

Ferric salts play a prominent role in the spectrophotometric determination of many pharmaceutical drugs. Acting as an oxidant, a ferric salt gets reduced to ferrous salt and this amount corresponds to drug concentration. The amount of Fe(II) can be determined using reagents such as 1,10-phenanthroline (PNL) and 2,2′-bipyridyl (BPL). These properties have been utilised to develop spectrophotometric methods for the determination of AMX, CPF and PIR.

3.1. Absorption spectra

AMX, CPF and PIR undergo oxidation by Fe(III) present in FPL and FBL. The Fe(II) so formed readily combines with PNL of FPL or BPL of FBL to form a red coloured complex, $[Fe(phen)_3]^{2+}$, having absorption maximum at 510 nm or $[Fe(bipy)_3]^{2+}$ exhibiting absorption maximum at 522 nm. Under the experimental conditions each reagent blank showed a negligible absorbance at the corresponding λ_{max}. The absorption spectra of coloured complexes for PIR are shown in Fig. 1.

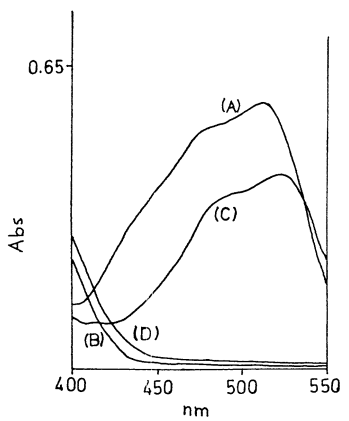

Fig. 1. Absorption spectra of (A) PIR (5 μg ml^{-1})–FPL system, (B) FPL reagent blank, (C) PIR (3.5 μg ml^{-1})–FBL system, and (D) FBL reagent blank.

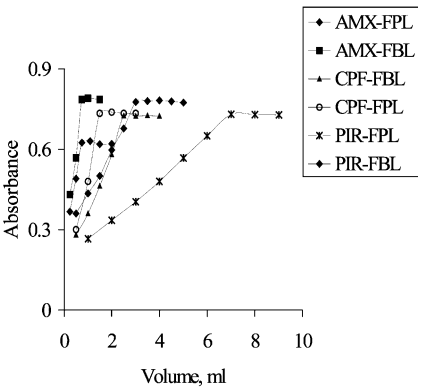

Fig. 2. Effects of reagents on the absorbances of complexes.

3.2. Optimum reaction conditions

The optimum reaction conditions for the quantitative determination of AMX, CPF and PIR were established via a number of preliminary experiments. The effects of the reagents were studied by measuring the absorbances of solutions containing a fixed concentration of AMX or CPF or PIR and varied amounts of the reagent separately. Constant and maximum colour development of the complex was achieved with a volume of 3, 1.5 and 7.0 ml of FPL for AMX, CPF and PIR, respectively, or with FBL volume of 0.75 ml for AMX and PIR, and 2.5 ml for CPF (Fig. 2). Although a larger volume of the reagent had no effect on the complex formation, the absorbances increased slightly due to background of the coloured reagent. However, 4, 2 and 8 ml of FPL for AMX, CPF and PIR, respectively, or a FBL volume of 1 ml for AMX and PIR, and 3 ml for CPF was used to ensure complete reaction.

The formation of coloured complex was slow at room temperature and required longer time for completion. Hence efforts were made to accelerate by carrying out the reaction at higher temperatures. It was observed that the maximum absorbances were obtained after heating the reaction mixture at 80 °C (20 min for AMX, 15 min for CPF), at 70 °C (5 min for PIR in method B) or allowed to stand at room temperature (25 min for PIR in method A). The absorbances of the complexes remained constant at room temperature for more than 24 h.

3.3. Quantification

In order to investigate the range in which the coloured complexes adhere to Beer's law, the absorbances of the complexes were measured at their respective λ_{max} values after developing the colour by following the suggested procedures for a series of solutions containing increasing amounts of the selected drugs. The Beer's law limits, molar absorptivity and Sandell's sensitivity values were evaluated and are given in Table 1. Regression analyses of Beer's law plots at their respective λ_{max} values revealed a good correlation. Graphs of absorbances versus concentration showed zero intercept, and are described by regression equation $Y = aX + b$ (where Y is the absorbance of a 1 cm layer, a is the slope, b is the intercept and X is the concentration of each of the selected drug in μg ml^{-1}) obtained by least-squares method. The results are summarised in Table 1.

3.4. Interference studies

The effects of common excipients and additives were tested for their possible interferences in the assay of AMX, CPF and PIR. It was observed that the talc, glucose, starch, lactose, sulphate, dextrose, acetate and magnesium stearate did not interfere in the determination at the levels found in dosage forms.

The recovery technique was applied to judge the suitability of the proposed methods. For this, known quantities of pure AMX, CPF and PIR solution were mixed with definite amounts of pre-analysed formulations and the mixtures were analysed as before. The total amount of the drug was then determined using the proposed methods and the amount of the added drug was calculated by difference. The results were found to be satisfactory.

3.5. Analysis of practical samples and statistical comparison of the results with official methods [1,2,4,5,36]

The proposed methods were successfully applied to the analysis of AMX, CPF and PIR in tablets and capsules. The results obtained were compared statistically by Student t-test and by the variance ratio F-test with those obtained by official methods. The Student t-values at 95% confidence level did not exceed the theoretical value indicating that there was no significant difference between the proposed and official methods. It was also observed that the variance ratio F-values

Table 1
Optical characteristics, precision and accuracy data

Parameter	Values of					
	Method A			Method B		
	AMX	CPF	PIR	AMX	CPF	PIR
λ_{max} (nm)	510	510	510	522	522	522
Beer's law limits (μg ml^{-1})	0.06–5.2	0.04–7.2	0.2–6.5	0.05–8.5	0.05–9.0	0.05–6.5
Molar absorptivity (10^4 l mol^{-1} cm^{-1})	14.6	3.4	3.96	4.14	2.95	3.78
Sandell's sensitivity (ng cm^{-2})	2.48	9.69	8.35	8.82	11.21	8.75
Correlation coefficient	0.9992	0.9990	0.9993	0.9995	0.9991	0.9989
Regression equation (Y)[a]						
Slope, a	0.0755	0.0778	0.1124	0.0756	0.0737	0.1122
Intercept, b	0.0901	0.0350	0.0213	0.0096	0.0645	0.0320
Relative standard deviation[b]	0.94	0.95	0.93	0.91	0.89	0.88
% Range of error[b] (95% confidence limit)	0.76	0.71	0.79	0.73	0.82	0.74

[a] $Y = aX + b$, where X is the concentration of drug in μg ml^{-1}.
[b] Average of five determinations.

Table 2
Determination of AMX, CPF and PIR in pharmaceutical preparations by the proposed methods and their comparison with official methods [1–5,36]

Drug	Label claim (mg per tablet or capsule)	Recovery[a] ± SD, % and their comparison with official methods		
		Official method	Method A	Method B
AMX				
Amokid tablet	250	99.86 ± 1.04	99.14 ± 0.85; $F = 1.49$; $t = 1.55$	99.15 ± 0.89; $F = 1.36$; $t = 1.67$
Biomox tablet	250	98.68 ± 0.91	99.12 ± 1.04; $F = 1.30$; $t = 1.61$	98.94 ± 0.68; $F = 1.79$; $t = 1.55$
Amoxil capsule	250	99.12 ± 1.08	99.64 ± 0.91; $F = 1.40$; $t = 1.79$	99.32 ± 0.84; $F = 1.65$; $t = 1.27$
Amyn capsule	250	98.97 ± 0.74	99.51 ± 0.88; $F = 1.41$; $t = 1.84$	99.10 ± 0.98; $F = 1.75$; $t = 1.37$
CPF				
Abact tablet	250	99.34 ± 0.85	98.93 ± 0.64; $F = 1.76$; $t = 1.95$	98.86 ± 0.71; $F = 1.15$; $t = 1.96$
Ciproace tablet	250	99.51 ± 0.89	99.43 ± 0.65; $F = 1.87$; $t = 1.78$	99.71 ± 1.02; $F = 1.31$; $t = 1.85$
Recipro capsule	250	98.91 ± 1.08	99.23 ± 0.81; $F = 1.77$; $t = 1.85$	99.84 ± 0.78; $F = 1.91$; $t = 1.48$
Recocif capsule	250	99.57 ± 0.86	99.46 ± 0.63; $F = 1.87$; $t = 1.59$	99.67 ± 0.69; $F = 1.55$; $t = 1.75$
PIR				
Falcam DT tablet	20	99.15 ± 0.84	98.88 ± 1.06; $F = 1.59$; $t = 1.74$	99.17 ± 0.71; $F = 1.39$; $t = 1.84$
Flexar DT tablet	20	98.93 ± 0.75	99.13 ± 1.01; $F = 1.81$; $t = 1.16$	98.95 ± 0.85; $F = 1.28$; $t = 1.65$
Amida capsule	20	98.89 ± 1.05	99.29 ± 0.84; $F = 1.31$; $t = 1.35$	99.54 ± 0.79; $F = 1.76$; $t = 1.91$
Brexic capsule	20	99.06 ± 0.87	99.64 ± 0.69; $F = 1.58$; $t = 1.94$	99.54 ± 1.08; $F = 1.54$; $t = 1.81$

[a] Average of five determinations.

calculated for $P = 0.05$ did not exceed the theoretical value (Table 2), indicating that there was no significant difference between the precision of the proposed and official methods.

4. Conclusion

The reagents provide fairly high sensitivity compared with most of the reagents reported earlier for the assay of AMX, CPF and PIR. The proposed methods are simple, accurate and economical with reasonable precision and accuracy. The FPL method was found to be more sensitive compared to FBL method for the assay of AMX, CPF and PIR. The coloured complexes are stable for more than 24 h, which makes the methods more practicable. The validity of the proposed methods is well demonstrated by analysing various dosage forms of AMX, CPF and PIR. Moreover, the methods are free from interference by common additives and excipients. These merits, in addition to the use of simple reagents, suggest their utility for routine quality control.

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