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Selective spectrofluorimetric determination of phenolic β-lactam antibiotics through the formation of their coumarin derivatives

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Abstract

A simple, sensitive and selective spectrofluorimetric procedure was developed for the determination of amoxycillin, cefadroxil and cefoperazone. The method is based on the reaction between these drugs and ethyl acetoacetate, in acidic medium, to give yellow fluorescent products with excitation wavelengths ranging from 401 to 467 nm and emission wavelengths ranging from 465 to 503 nm. The reaction conditions were studied and optimized. The reaction obeyed Beer's law over the range of 10.0–20.0, 1.5–1.0 and 50.0–100.0 μg ml^{-1} for amoxycillin, cefadroxil and cefoperazone, respectively. Interference's from other antibiotics, drugs and dosage forms additives, in capsules and vials dosage forms, were investigated. The proposed method was applied to the analysis of pharmaceutical formulations (capsules and vials) containing the above antibiotics, either alone or in combination with other antibiotics or drugs. The validity of the method was tested by the recovery studies of standard addition which were found to be satisfactory. The results of the proposed method demonstrated that the method is equally accurate, precise and reproducible as the official methods (USP XXIII) and those published for the non-official binary mixtures.

Keywords: Amoxycillin; Cefadroxil; Cefoperazone; Ethyl acetoactate; Spectrofluorimetry; Capsules; Vials

1. Introduction

Amoxycillin trihydrate or its sodium salt are one of the most widely used penicillins either alone or in combination with other drugs. For a broader spectrum of antibacterial activity, pharmaceutical preparations containing binary mixtures of amoxycillin with potassium clavulanate, flucloxacillin, cloxacillin and dicloxacillin are found on the market [1]. For more combined therapeutic action, other drugs than penicillins, such as probenecid; trimethoprim; bromohexine; ambroxol and carbocysteine, are combined with amoxycillin in pharmaceutical dosage forms [2]. Cefadroxil monohydrate and cefoperazone represent the first and the third generations from the

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semisynthetic cephalosporins. The three antibiotics are the only available phenolic antibacterials. Cefadroxil and cefoperazone are, mostly, dispensed as a single component. The chemical and pharmacological properties of the antibacterials, in general, have been widely explored because of their extensive medical applications [3].

Several analytical techniques are available in the literature for the determination of these antibiotics in their pharmaceutical preparations. The three antibiotics are official in the USP XXIII [4], while only amoxycillin is official in the BP 1993 [5]. The third edition of the *European Pharmacopoeia* contains two monographs for amoxycillin and cefadroxil [6]. The BP 1993 gives a mercurimetric titration method for the assay of amoxycillin in bulk drug and direct UV–spectrophotometric method for the pharmaceutical dosage forms. The USP XXIII gives three HPLC procedures for the determination of the three antibacterials in bulk drug and in their pharmaceutical dosage forms. The binary mixture of amoxycillin and potassium clavulanate is official, only, in the USP XXIII. The physical and chemical characteristics of amoxycillin with some references for its quantitation have been collected in two profiles [7,8]. The determination of amoxycillin in its pharmaceutical preparations was based on iodometric and mercurimetric titrations [9], fluorimetry [10] and polarography [11,12]. Many HPLC procedures have been published [13–16]. Several colorimetric procedures based on the treatment with paramolybdate anion [17], 2, 3-dichloro - 5,6 - dicyano - *p* - benzoquinone [18], sodium cobaltinitrite [19], ninhydrin [20], chloranil [21],3,5-dinitrobenzoic acid [22] and *N*-halosuccinimide [23] have been reported. Derivative spectrophotometric methods have been reported for the determination of amoxycillin in the presence of potassium clavulanate [24], dicloxacillin [25] and flucloxacillin [26]. On the other hand, cefadroxil was determined colorimetrically [23,27,28], liquid chromatographically [29–31] and using a continuous flow injection technique [32]. The determination of cefoperazone was possible by colorimetry [33,34], adsorptive stripping voltammetry [35], differential pulse polarography [36] and liquid chromatography techniques [37–39]. The zero-crossing derivative spectrophotometric technique had been used for the quantitation of a theoretical binary mixture of cefoperazone with cefamandole [40] and sulbactam [41]. As the official pharmacopoeia procedures for the determination of the above-mentioned antibacterials involve the use of expensive equipment and reagents, it was considered worthwhile to develop a rapid and selective procedure suitable for application in quality control laboratories. The aim of the present work is to develop a simple, direct and selective spectrofluorimetric procedure for the determination of amoxycillin trihydrate or its sodium salt, cefadroxil monohydrate and cefoperazone in single and multi-component pharmaceutical dosage forms. The proposed method is based on the reaction between the phenolic antibiotics and ethyl acetoacetate in the presence of a dehydrating agent (von Pechmann–Duisberg condensation) [42,43] and the subsequent measurement of the fluorescence of the compounds formed. Hitherto,

Table 1
Optimum conditions for the fluorescence development

Parameter	AM	CD	CP
Volume of reagent (ml)	1.0	0.5	0.5
Volume of the acid (ml)	2.5	3.5	2.5
Temperature (°C)	80	Boiling water	60
Heating time (min)	5	5	15

Table 2
Fluorescence characteristics and quantitative parameters for the formed coumarins

Parameter	AM	CD	CP
Wavelength of excitation (nm)	467	401	412
Wavelength of emission (nm)	503	485	465
Beer's law limit (μg ml^{-1})	10–20	1.5–7.0	50–100
Regression equations			
Intercept (a)	3.10	1.21	0.74
S_a	0.2007	0.2416	0.1555
Slope (b)	0.85	9.72	0.19
S_b	0.0156	0.0674	0.0024
Correlation coefficients (r)	0.9995	0.9999	0.9999
Variances (S_0^2)	0.0167	0.0733	0.01
Detection limits (μg ml^{-1})	0.4200	0.0767	1.45

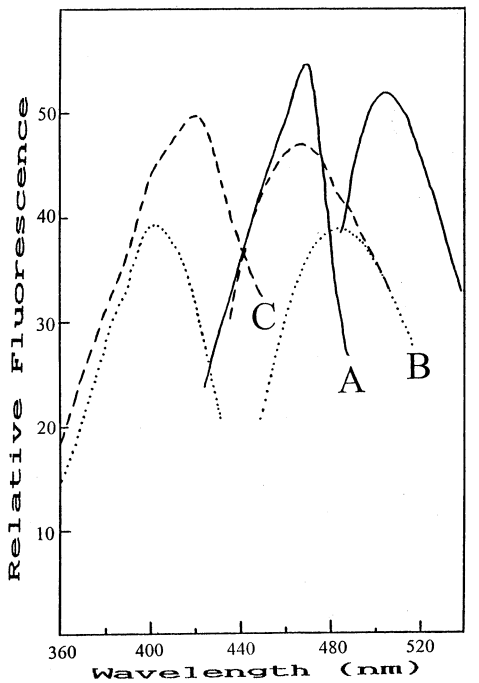


Fig. 1. Excitation and emission spectra of (A) amoxycillin (20 μg ml^{-1}) (—), (B) cefadroxil (1.60 μg ml^{-1}) (···) and, (C) cefoperazone (80 μg ml^{-1}) (---) as their reaction product with ethyl acetoacetate.

no reports of the use of this reaction for the determination of phenolic drugs was reported in the literature. The method was applied to the determination of amoxycillin, cefadroxil and cefoperazone in both pure form and pharmaceutical formulations.

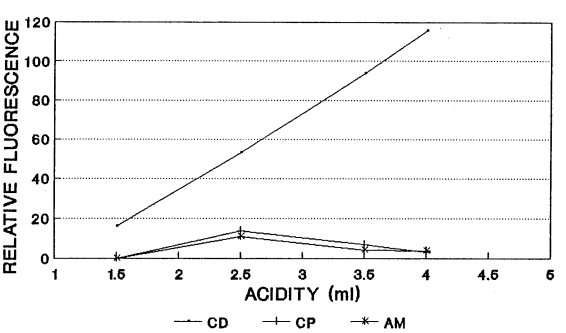


Fig. 2. Effect of the acidity (volume of sulfuric acid) on the fluorescence of amoxycillin (10 μg ml^{-1}, *), cefadroxil (10 μg ml^{-1}, ●) and cefoperazone (60 μg ml^{-1}, +).

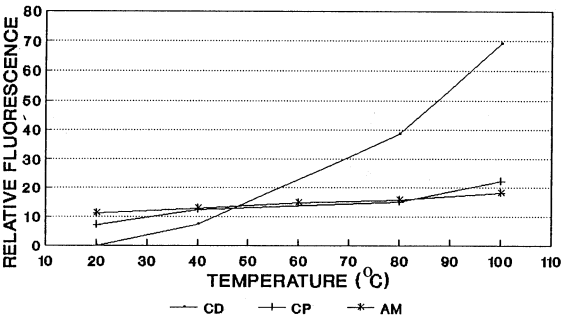


Fig. 3. Effect of the temperature on the fluorescence intensity of amoxycillin (10 μg ml^{-1}, *), cefadroxil (5 μg ml^{-1}, ●) and cefoperazone (60 μg ml^{-1}, +) at ambient temperature, 40, 60, 80°C and boiling water bath.

A statistical comparison of the results was made with either the official methods, in single component form, or by adopting the published procedure for the binary mixtures. The results showed that the proposed procedure compared favorably with the officials methods and satisfactory accuracy and precision were noted. Other advantages of the method are its simplicity and relative speed.

2. Experimental

2.1. Materials and reagents

Both amoxycillin (AM) and cefadroxil (CD) were obtained from Pharco Pharmaceuticals Co.

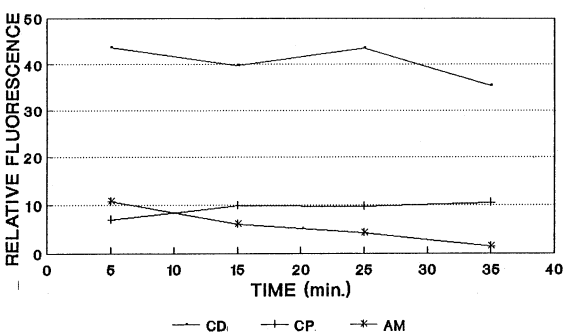


Fig. 4. Effect of the heating time on the fluorescence intensity of amoxycillin (10 μg ml^{-1}, *), cefadroxil (3 μg ml^{-1}, ●) and cefoperazone (60 μg ml^{-1}, +).

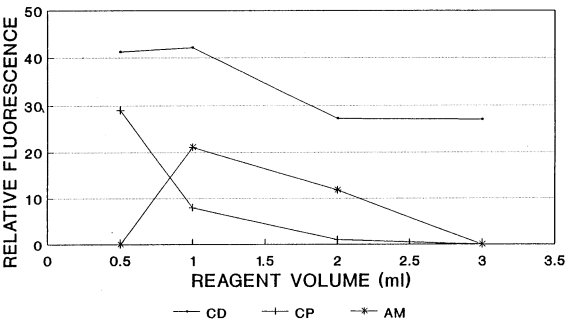


Fig. 5. Effect of the reagent (ethyl acetoacetate) concentration on the fluorescence intensity of amoxycillin (10, μg ml^{-1}, *) cefadroxil (10 μg ml^{-1}, ●) and cefoperazone (100 μg ml^{-1}, +).

(Alexandria, Egypt) and their potencies were certified to contain 960 and 975 mg g^{-1}, respectively. Cefoperazone was donated by Pfizer Egypt (Cairo, Egypt) and was labeled to contain 970 mg g^{-1}. Ethyl acetoacetate (Prolabo, Paris, France) was distilled twice before use. The reagent was prepared as 2% (v/v) solution in absolute ethanol and should be freshly prepared. Sulfuric acid (BDH, Poole, UK) was labeled to contain 98%.

2.2. Apparatus

The fluorescence intensities were measured on a Perkin–Elmer Model 560-10S spectrophotometer equipped with a 150 W xenon lamp, excitation, emission grating monochromators and 1 × 1 cm quartz cell and attached to a Perkin–Elmer

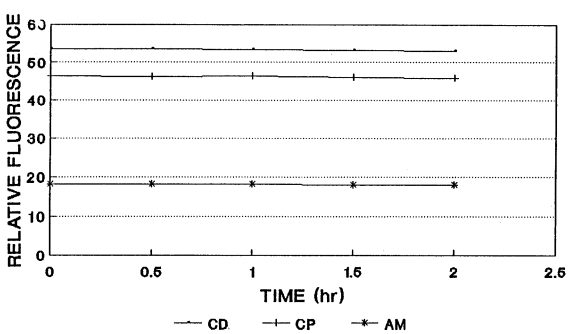
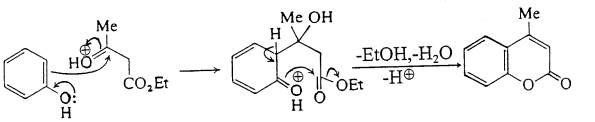


Fig. 6. Fluorescence stability at ambient temperature for the formed coumarins of amoxycillin (18 μg ml^{-1}, *), cefadroxil,(3 μg ml^{-1}, ●), and cefoperazone (70 μg ml^{-1}, +).



Scheme 1.

Model 56 recorder. The spectrofluorimeter was a gift from the Alexander von Humboldt Foundation (Bonn, Germany) to Prof. A.M. Wahbi. A thermostatted water bath, accurate to ±0.5°C, was utilized throughout. The official USP XXIII HPLC procedures for the analysis of the three antibacterials were performed using a Hewlett–Packard system (Avondale, PA, USA) Model 1090 LC equipped with a diode-array detector and controlled using HP Chem station. The methods used a C_{18} reversed phase column (25 cm × 4.6 mm ID) with a slight modification in the mobile phase.

2.3. Preparation of the standard solutions

Stock solutions of AM, CD and CP were prepared by dissolving 100 mg from each powder in the minimum amount of methanol (3 ml) in a 100 ml volumetric flask and diluting to volume with absolute ethanol. Further dilutions were made to give final concentrations of 0.1, 0.05 and 0.5 mg ml^{-1} for AM, CD and CP solutions, respectively. The solutions were stable for at least 72 h, if they had been stored in a cool and dark place.

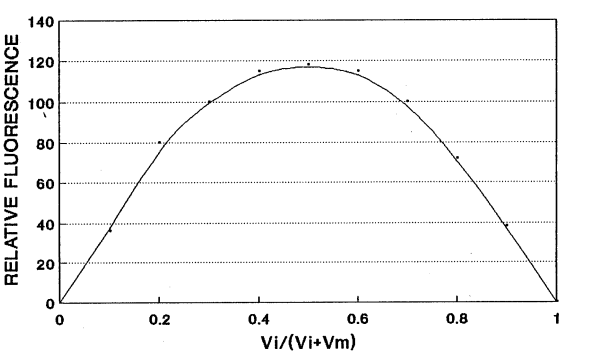


Fig. 7. Continuous variation graph of phenol-ethyl acetoacetate, (1 × 10^{-3} M).

Table 3
Evaluation of the accuracy and precision of the proposed method for the determination of amoxycillin (AM), cefadroxil (CD) and cefoperazone (CP)

Drug	Added[a]	Found ± SD[b]	RSD%[c]	SAE[d]	Confidence limits[e]
AM	1.0	1.00 ± 0.008	0.836	3.74E−3[f]	100 ± 0.010
	1.4	1.42 ± 0.017	1.179	1.47E−3	1.42 ± 0.021
	2.0	2.00 ± 0.008	0.418	3.74E−3	2.00 ± 0.010
		Mean	0.811	4.98E−3	
CD	0.15	0.15 ± 0.001	0.672	4.52E−4	0.15 ± 0.001
	0.35	0.35 ± 0.001	0.386	6.04E−4	0.35 ± 0.002
	0.70	0.70 ± 0.001	0.210	6.57E−4	0.70 ± 0.002
		Mean	0.423	5.75E−4	
CP	5.00	4.97 ± 0.043	0.869	1.90E−2	4.97 ± 0.053
	7.00	7.00 ± 0.024	0.345	1.10E−2	7.00 ± 0.031
	9.00	9.00 ± 0.031	0.339	1.40E−2	9.00 ± 0.039
		Mean	0.518	1.50E−2	

[a] Final concentration in mg per 100 ml.
[b] Mean ± SD for five determinations.
[c] RSD%, relative standard deviation.
[d] SAE, standard analytical error.
[e] Confidence limits at $P = 0.05$ and four degrees of freedom.
[f] E−3 = $\times 10^{-3}$.

2.4. Construction of the calibration graphs

To three sets of 10 ml volumetric flasks, different concentrations from the stock solutions of the three antibacterials were quantitatively transferred. Suitable volumes of ethyl acetoacetate and sulfuric acid were added (Table 1) to each flask. The reaction mixtures were heated on water bath at the proper temperature for the specified time stated in Table 1. The flasks were then cooled and completed to volume with ethanol. The fluorescence of the resulting solutions were measured at the specified excitation and emission wavelengths (Table 2). A blank was prepared, by omitting the drug, and its fluorescence was measured simultaneously.

2.5. Preparation of the commercial samples

2.5.1. For capsules

The contents of ten capsules were emptied, as completely as possible, and weighed. An accurately weighed portion of the powder, equivalent to 100 mg of each antibacterials, was transferred to a 100 ml volumetric flask with the aid of 3 ml methanol. The flasks were filled to volume using absolute ethanol. The contents of each flask were mixed for 10 min with the aid of a magnetic stirrer. The mixtures were filtered and the first 5 ml of the filtrate was discarded. The clear filtrates were then diluted with absolute ethanol to suit the procedure mentioned above.

2.5.2. For tablets

Ten tablets were powdered and a quantity of the powder equivalent to 100 mg of the antibacterials was transferred quantitatively to 100 ml volumetric flask with the help of 3 ml methanol. The procedure was completed as described in Section 2.5.1.

2.5.3. For vials

The powder contents of a vial was transferred quantitatively to a 100 ml volumetric flask with the help of 3 ml methanol and subsequent dilution was made with absolute ethanol. The procedure was completed as mentioned in Section 2.5.1.

Table 4
Analysis of amoxycillin (AM), cefadroxil (CD) and cefoperazone (CP) in some commercial pharmaceutical preparations

Preparation name	Recovery ± SD	
	Proposed method[a]	Reference method[b]
Amoxycillin preparations[c]		
I. *Capsules and tablets*		
Amoxil (BN 970811)	96.19 ± 0.61	96.06 ± 0.40
	$t = 0.34$ (2.31)[d]	$F = 2.33$ (6.39)[e]
Amoxycillin (BN 702036)	97.88 ± 0.49	97.96 ± 0.64
	$t = 0.25$	$F = 1.71$
Recovery[f]	99.95 ± 0.55	100.00 ± 0.60
	$t = 0.14$	$F = 1.19$
Amoxicid (BN 695102)	100.30 ± 0.68	100.20 ± 0.39
	$t = 0.23$	$F = 3.04$
E-Mox (BN 972225)	97.63 ± 0.54	97.45 ± 0.35
	$t = 0.53$	$F = 2.38$
Flemoxin (BN 805125)	99.35 ± 0.62	99.65 ± 0.98
	$t = 0.77$	$F = 2.50$
Recovery	100.49 ± 0.47	100.01 ± 0.94
	$t = 1.16$	$F = 4.00$
Hiconcil (BN 330697)	99.59 ± 0.65	99.29 ± 0.91
	$t = 0.73$	$F = 1.96$
Ibiamox (BN 842)	98.02 ± 0.13	97.93 ± 0.22
	$t = 1.09$	$F = 2.86$
Ospamox (BN 93564)	99.85 ± 0.77	100.15 ± 0.96
	$t = 0.62$	$F = 1.55$
Recovery	100.05 ± 0.85	100.20 ± 0.90
	$t = 0.28$	$F = 1.12$
II. *Vials*		
E-Mox (BN 963592)	98.93 ± 0.18	99.01 ± 0.19
	$t = 0.70$	$F = 1.11$
Farconcil (BN 101T)	101.49 ± 0.47	101.01 ± 0.94
	$t = 1.61$	$F = 4.00$
Recovery	99.95 ± 0.96	100.05 ± 1.20
	$t = 0.16$	$F = 1.56$
Cefadroxil Capsules[g]		
Curisafe (BN 370497)	100.62 ± 1.05	100.25 ± 0.96
	$t = 0.56$	$F = 1.20$
Duricef (BN J71986)	101.46 ± 1.60	101.20 ± 1.25
	$t = 0.26$	$F = 1.63$
Recovery	100.25 ± 0.85	100.05 ± 0.99
	$t = 0.37$	$F = 1.64$
Ibidroxil (BN 1898)	102.39 ± 0.48	102.32 ± 0.79
	$t = 0.23$	$F = 2.71$
Cefoperazone Vials[h]		
Cefobid (BN 72535102)	99.92 ± 0.72	100.39 ± 0.39
	$t = 1.03$	$F = 3.41$
Cefoperazone (BN10B)	100.40 ± 0.80	100.40 ± 0.77
	$t = 0.00$	$F = 1.55$
Cefazone (BN 100D)	100.38 ± 0.92	99.81 ± 0.74
	$t = 0.98$	$F = 1.54$
Recovery	100.05 ± 0.45	100.15 ± 0.60
	$t = 0.35$	$F = 1.78$

[a] Mean ± SD of five determinations.

[b] USP XXIII, Mean and SD of five determinations.

[c] All the amoxycillin capsules which were labeled to contain 500 mg per capsule, except Ospamox tablets were labeled to contain 1000 mg per tablet. Amoxil capsules manufactured by Medical Union Pharmaceutical Co., Abu Sultan, Egypt, under license from Beecham Pharmaceuticals, Brentford, England; Amoxycillin capsules manufactured by the Arab Drug Co., Cairo, Egypt; Amoxicid capsules manufactured by Chemical Industries Development Co., Giza, Egypt; E-Mox capsules manufactured by the Egyptian International Pharmaceutical Industries Co., 10th of Ramadan City, Egypt; Hiconcil capsules manufactured by Pharco Pharmaceuticals Co., Alexandria, Egypt, under licensee from BMS, USA; Ibiamox capsules manufactured by AMOUN Pharmaceutical Co., El-Salam City, Egypt, under license from IBI, Milano, Italy; Ospamox tablets manufactured by October Pharma, 6 October City, Egypt, under license from Biochemie, Austria.

[d] Tabulated t-value for $P = 0.05$ and 8 degree of freedom; the t-values were calculated according to the equation in [55].

[e] Tabulated F-value for $P = 0.05$ and $f_1 = f_2 = 4$.

[f] Standard addition method.

[g] All cefadroxil capsules were labeled to contain 500 mg per capsule; Curisafe capsules manufactured by Pharco Pharm. Co., Alexandria, Egypt; Duricef capsules manufactured by Bristol-Meyers Squibb, Egypt; Ibidroxil capsules manufactured by Amoun Pharm. Co., El-Salam City, Egypt.

[h] All cefoperazone vials were labeled to contain 1 g per vial, Cefobid vials manufactured by Pfizer Egypt, under the authority of Pfizer Inc., USA; Cefoperazone vials manufactured by CID for the T3A Pharm. Co.; Cefazone vials manufactured by Pharco Pharm. Co., Alexandria, Egypt.

3. Results and discussion

Phenolic compounds are known to condense with ethyl acetoacetate (EAA) in the presence of concentrated sulfuric acid, as dehydrating agent, in absolute ethanol, to give yellow fluorescent coumarins [42,43]. The above-mentioned reaction is known as the von Pechmann–Duisberg condensation. Amoxycillin, cefadroxil and cefoperazone, which are three phenolic compounds among the available β-lactam antibiotics, were found to be a good candidate for the application of the above condensation reaction. The three antibacterials reacted with ethyl acetoacetate and gave an intense yellow fluorescence with excitation and emission wavelengths ranging from 401 to 467 nm and from 465 to 503 nm, respectively (Fig. 1 and Table 2). Such yellow fluorescence was not observed on carrying out the reaction with other β-lactams (potassium clavulanate, cloxacillin, dicloxacillin and flucloxacillin) and drugs (probenecid, ambroxol, bromohexine, trimethoprim and carbocisteine). At the same time, ethyl acetoacetate in the acidic medium has no fluorescence, in the above-mentioned region.

3.1. Optimization of the reaction conditions

The reaction conditions with respect to the reaction medium, the reagent concentration, the acidity or the volume of the sulfuric acid and reaction time and temperature were optimized to achieve maximum sensitivity. Our first goal was to choose the most appropriate solvent for this type of condensation. The fluorescence of the solutions as a function of the solvents was compared. Solvents investigated include absolute ethanol, 50% aqueous ethanol, acetonitrile, acetone and dioxan. Of all the solvents, the highest fluorescence was obtained upon using absolute ethanol. This proves that this type of condensation reaction has to be carried out in a completely anhydrous medium.

The fluorescence of the solutions was investigated over the sulfuric acid volume range of 1.5–4.5 ml. The optimum fluorescence was achieved at 2.5, 4.5 and 2.5 ml sulfuric acid for AM, CD and CP, respectively (Fig. 2). The effect of the acidity of the medium was studied at the specified excitation and emission wavelengths stated in Table 2. In order to examine the effect of temperature and heating time on the fluorescence of the formed coumarins, the condensation reactions were carried out at different temperatures [ambient temperature (22°C), 40, 60, 80°C and boiling water bath] using a thermostatted water bath (Fig. 3) for periods ranging from 5 to 35 min (Fig. 4). The appropriate temperature and heating time for each antibacterial are illustrated in Table 1. The effect of the reagent concentration (volume of 1% ethanolic solution of ethyl acetoacetate) on the resulting fluorescence was studied (Fig. 5). The optimum fluorescence was obtained using 1.0, 1.0 and 0.5 ml of the reagent solution for AM, CD and CP, respectively. Finally, water, ethanol, acetone and isopropanol were tested as diluting solvents. The results obtained revealed that ethanol, methanol and isopropanol were the best solvents for the procedure. Water and other inmiscible solvents (e.g. chloroform) were not suitable, therefore, ethanol was chosen as the diluting solvent. It was noticed that the fluorescence intensity increased in highly acidic medium and with raising the heating temperature. Unfortunately, this was coupled with an increase in the background reading, which means less relative fluorescence intensity. Therefore, the selected parameters did not give, by necessity, the highest fluorescence intensity to avoid drastic conditions and high background readings. The fluorescence of the formed coumarins under the above-mentioned optimum conditions was stable for at least 1 h (Fig. 6).

3.2. The reaction mechanism

A general synthesis of coumarins involves the interaction of phenol with a β-ketoester (ethyl acetoacetate) in the presence of a condensing or dehydrating agent. Concentrated sulfuric acid is generally used as condensing and dehydrating agent for simple monohydric phenol [44]. The highly reactive ethyl acetoacetate reacts with the three investigated phenolic β-lactam antibiotics in the presence of sulfuric acid to cyclize into a coumarin ring and form a yellow fluorescent product. The mechanism of the reaction is

thought to involve the initial formation of a β-hydroxyester, which then cyclizes and dehydrates to yield the coumarin (Scheme 1). In order to study the reaction further, simple phenol was chosen as a model to determine the molar ratio of the ethyl acetoacetate to the phenol. The yellow fluorescence of the formed 3-methyl coumarin was found to have excitation and emission wavelengths of 365 and 425 nm, respectively. The highest fluorescence of the phenol was obtained when using 1 ml of ethyl acetoacetate reagent, 2.5 ml sulfuric acid and heating the solution in a boiling water bath for 15 min [45]. Application of Job's method of continuous variation [46] using equimolar solutions of phenol and ethyl acetoacetate (1×10^{-3} M) revealed a 1:1 molar ratio as illustrated in Scheme 1 and Fig. 7.

3.3. Validation

Validation was carried out specifically on the current application of the methodology to the three β-lactam antibiotics.

Table 5
Determination of amoxycillin (250 mg per capsule or vials) in commercial binary mixtures with other penicillins

Preparation name[a]	Co-existing penicillin	Recovery ± SD[b]	
		Proposed method	Reference method
I. Capsules and tablets			
Amoclox	Dicloxacillin	100.53 ± 0.45	100.56 ± 0.66[c]
(BN 802041)		$t = 0.11$	$F = 2.15$
Recovery		100.05 ± 0.86	99.95 ± 0.84[c]
		$t = 0.18$	$F = 1.05$
Miclox	Dicloxacillin	98.41 ± 1.37	99.45 ± 0.72[c]
(BN 811125)		$t = 1.20$	$F = 3.62$
Flumox	Flucloxacillin	100.30 ± 0.62	99.85 ± 1.00[d]
(BN 975149)		$t = 1.15$	$F = 2.60$
Recovery		99.75 ± 0.65	100.00 ± 0.60[d]
		$t = 1.64$	$F = 1.17$
Flucamox	Flucloxacillin	99.10 ± 1.45	99.90 ± 1.75[d]
(BN 697149)		$t = 0.87$	$F = 1.46$
Hi-Flucil	Flucloxacillin	100.10 ± 0.55	100.25 ± 0.80[d]
(BN 397102)		$t = 0.43$	$F = 2.11$
Augmentin	Pot. Clavulanate	100.45 ± 0.76	100.25 ± 0.87[e]
(BN 970583)		$t = 0.42$	$F = 1.31$
Recovery		100.20 ± 0.85	100.40 ± 0.70[e]
		$t = 0.37$	$F = 1.47$
II. Vials			
Flumox	Flucloxacillin	98.85 ± 1.20	99.15 ± 1.00[d]
(BN 973168)		$t = 0.39$	$F = 1.44$
Recovery		100.25 ± 0.75	100.00 ± 0.85[d]
		$t = 0.53$	$F = 1.28$

[a] All the binary mixtures were labeled to contain 250 mg from each of amoxycillin and dicloxacillin or flucloxacillin. Amoclox capsules manufactured by Memphis Chemical Co., Cairo, Egypt; Miclox capsules manufactured by Misr Co. for Pharmaceutical Industries, Cairo, Egypt; Flumox capsules and vials manufactured by Egyptian International Pharmaceutical Industries Co., 10th of Ramadan City, Egypt; Flucamox capsules manufactured by South Egypt Drug Industries Co., 6 October City, Egypt; Hi-Flucil capsules manufactured by Nile Co. for Pharmaceuticals and Chemical Industries, Cairo, Egypt.

[b] Mean and SD of five determinations.

[c] Ref. [49].

[d] Ref. [48].

[e] Ref. [47].

Table 6
Determination of amoxycillin and cefoperazone in synthetic binary mixtures with other drugs

Co-existing drug[a]	Dose (mg)	Recovery ± SD[b]	
		Proposed method	Reference method
Amoxycillin trihydrate 250 mg			
Ambroxol HCl	15	100.54 ± 0.77	99.88 ± 0.67 [52][c]
		$t = 1.36$	$F = 1.32$[d]
Bromohexine HCl	8	100.20 ± 0.58	99.85 ± 0.95 [52]
		$t = 0.95$	$F = 2.68$
Carbocisteine	150	99.75 ± 1.00	99.55 ± 0.85 [50]
		$t = 0.32$	$F = 1.38$
Cloxacillin	250	100.10 ± 0.58	100.15 ± 0.98 [51]
		$t = 0.14$	$F = 2.85$
Probenecid	200	99.80 ± 1.20	100.05 ± 1.15 [52]
		$t = 0.33$	$F = 1.09$
Trimethoprim	80	100.30 ± 0.62	99.95 ± 0.75 [52]
		$t = 0.89$	$F = 1.46$
Cefoperazone (1 g in each mixture)			
Cefamandole	1000 g	100.40 ± 0.75	100.30 ± 0.85 [53]
		$t = 0.21$	$F = 1.28$
Sulbactam	500	99.95 ± 0.52	100.05 ± 0.70 [54]
		$t = 0.30$	$F = 1.81$

[a] Due to the unavailability of these binary mixtures commercially on the local market, synthetic mixtures were prepared in tablet matrix [56]; with 2% magnisum stearate in the presence of cloxacillin to prepare capsules and with no additives for cefoperazone vial mixtures.
[b] Mean and SD of five determinations.
[c] Reference number in the list.
[d] Critical values at $P = 0.95$, $t = 2.31$ and $F = 6.39$.

3.3.1. Linearity of the method

Under the specified reaction conditions (Table 1), the relative fluorescence intensity at the specified emission wavelengths (Table 2) was found to be proportional to the concentrations of the three investigated β-lactam antibiotics (AM, CD and CP). Table 2 shows the most important fluorescence characteristics and quantitative parameters, such as the slopes, the intercepts, the variances and the correlation coefficients obtained by the linear squares treatment of the results.

3.3.2. Specificity and interference studies

Due to the phenolic nature of the three β-lactam antibacterials, the reaction was found to be specific for them in the presence of other antibiotics or drugs not bearing a phenolic group with a free *ortho* position. Potassium clavulanate, sulbactam and other related penicillins and cephalosporins, such as flucloxacillin sodium, cloxacillin, dicloxacillin monohydrate and cefamandole were found not to interfere. Other drugs (trimethoprim, probenecid, ambroxol, bromohexine, carbocisteine) dispensed in binary mixtures with amoxycillin trihydrate do not interfere. It was also shown that excipients and diluents such as starch, talc, magnesim stearate, avicel or microcrystalline cellulose and titanium dioxide, which are commonly formulated in tablets and capsules dosage forms, do not interfere with the proposed method. Unfortunately, the oral suspension dosage form, which contains large amount of sugars, was found to interfere. The sugar charred immediately after heating the solution following the addition of the concentrated sulfuric acid. Many attempts were made to get rid of the sugar before carrying out the assay but without success. At the same time and because of the dependence of the reaction on the presence of a phenolic group attached to the β-lactam nucleus (5 or 6 membered rings) in the antibacterial molecules, the acidic and alkaline degradation products of three antibacterials were found to interfere.

3.3.3. Precision and accuracy

In order to determine the accuracy and the precision of the procedure, solutions containing three different concentrations of AM, CD and CP were prepared and analyzed in five replicates. The analytical results obtained from this investigation, are summarized in Table 3. The mean standard deviation (SD), the relative standard deviation (RSD%) and the mean standard error (SAE) can be considered adequate for the quality control analysis of pharmaceutical preparations.

3.4. Analysis of commercial pharmaceutical dosage forms

The proposed procedure was applied to the determination of AM and CP, either in single preparations or in binary mixtures with other penicillin's and drugs, as well as CD in dosage forms. Tables 4–6 show the results obtained for the determination of the drugs in pharmaceutical preparations by means of the proposed method and the reference methods. For comparisons, the three antibacterials single preparations were determined using the USP XXIII procedures and using reported procedures for the binary mixtures of AM [47–51] and CP [52,53]. These determinations were carried out on the same batch of samples. The results obtained were compared statistically by Student's t-test and variance ratio F-test (Tables 4–6). The experimental values did not exceed the theoretical ones in both tests which indicates that there were no significant differences between the methods compared. For more confirmation, the standard addition method was applied, to some commercial products, to test the reliability and recovery of the proposed method. The recovery studies were carried out after adding known quantities of pure drug to the preanalyzed formulations. The percentage recoveries were almost quantitative (100%) (Tables 4 and 5).

4. Conclusion

The coumarin derivatives formed under the above-mentioned conditions and measured spectrofluorimetrically can be regarded as sensitive, simple and rapid procedure for the determination of the phenolic antibacterials in bulk, capsules and vials dosage forms. The method had been validated for the determination of the phenolic antibacterials and was found to be significally faster and cheaper than the current procedures.

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