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Comparison of several methods used for the determination of cephalosporins. Analysis of cephalexin in pharmaceutical samples

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Abstract

The precision of UV absorbance of intac and acid degraded cephalosporins, ninhydrin, high performance liquid chromatography and iodometric methods used for analysis of cefoxitin, cefotaxime, cephazolin and cephalexin were compared. To obtain the calibration graphs the analytical signal used were: absorbance, first derivative absorbance, second derivative absorbance and H-point Standard Additions Method by using absorbance values at two selected wavelengths as analytical signal. These methods and calibration graphs were also used for the determination of cephalexin in pharmaceutical samples.

Keywords: Cephalosporins; Ninhydrin; Spectrophotometry; High performance liquid chromatography; H-point Standard Additions Method; Pharmaceutical samples

1. Introduction

β-Lactam antibiotics have been used since the discovery of penicillin in 1928. The penicillins and the cephalosporins are both β-lactam. Cephalosporins have been used since 1948. These antibiotics have assumed a prominent role in modern antimicrobial therapy due to enhanced intrinsic microbiological activities and favourable safety profile. The 7-aminocephalosporanic acid (7-ACA) (Fig. 1) proceeds from the hydrolysis of the cephalosporin C biologically active. Chemical structure of cephalosporins derive from the 7-ACA composed of a β-lactam ring fused with a dihydrothiazine ring (Fig. 1), but differ in the nature of substituents attached at the 3- and/or 7-positions of the cephem ring. These substitutions affect either the pharmacokinetic properties (3-position) or the antibacterial spectrum (7-position) of the cephalosporins [1]. Traditionally, the cephalosporins are divided into first-, second-, third- and fourth-generation agents [2,3]. Fig. 1 shows the cephalosporins studied in this work.

Several methods have been reported for cephalosporin determinations. The official procedures in pharmaceutical preparations utilise iodo-

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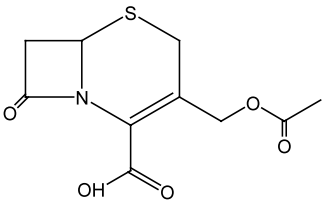
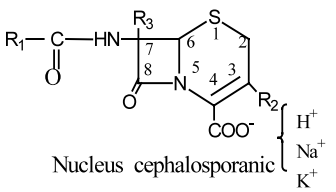


Structure of the 7-aminocephalosporanic acid.



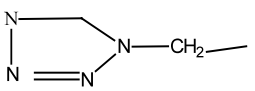
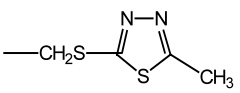
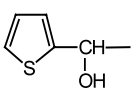
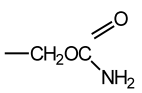
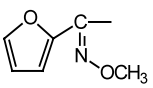
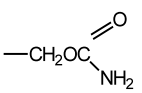
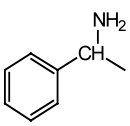
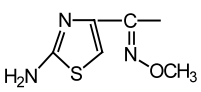
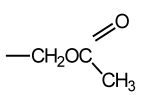
First generation	R_1	R_2	R_3
Cefazolin			H
Second generation			
Cefoxitin †			OCH_3
Cefuroxime			H
Cephalexine			H
Third generation			
Cefotaxime			H

Fig. 1. Structure of the 7-ACA and structure general of the studied cephalosporins.

metric titration [4,5], the microbial assay [6], spectrophotometric UV–vis analysis [7] and high-performance liquid chromatography (HPLC) methods [8]. The iodometric method is unselective as all antibiotics, the initial substances for their production as well as their degradation products, are oxidised by iodine. It is therefore not suitable for control of the purity of the antibiotics of this group. The direct spectrophotometric methods suffer a lack of specificity because all compounds containing the β-lactam ring absorb in the range 250–270 nm. The major drawback of microbiological procedures is their lack of specificity in stability studies when the decomposition products are microbiologically active [9]. In order to improve the selectivity and sensitivity the HPLC with ultraviolet absorption detection is the method of choice frequently used for cephalosporin determinations in pharmaceutical samples. In most formulations the added ingredients do not generally interfere with these methods.

Different reagents have been reported in order to increase the selectivity of the methods based on the own cephalosporins absorption or their products of acid or basic degradation. Table 1 summarises the main parameters of different proposed methods that use the spectroscopy UV–vis [10–32]. Hydroxylamine reacts with a number of carboxylic acid derivatives to form hydroxamic acids in presence of nickel (II) [10] or iron (III) [30] as catalyst in the cephalosporins determination. The presence of amides, esters, aldehydes, ketones, anhydrides, etc. which also react with hydroxylamine, leads to positive errors in this determination. The hydroxylamine method is more selective than iodometric or biological methods by less so than HPLC methods. The ninhydrin reagent [11,17] is known to condense with thiophene and some of its derivatives in the presence of concentrated sulphuric acid to yield a coloured product [33]. Moreover, ninhydrin reacts with the 2-thienylacetic acid formed as a degradation product of cephalotin (analogous cephalosporin of cefoxitin) in a strong sulphuric acid medium [11]. In such a medium, cefoxitin also suffers a hydrolytic process [23]. Spectrophotometric determination of cephaloridine, cefoxitin and cephalotin using ninhydrin in a strong sulphuric acid medium is based on the presence of an unsubstituted thienyl moiety in both the α- and β-positions [17]. The determination of cephalosporins by alkaline degradation to sulphide hydrogen and formation of methylene blue has been proposed [12]. Cephazolin [15] suffered acid degradation when is heated in the presence of sulphuric acid. This process can be observed by means of the disapparition of the band of 270 nm and the apparition of a new band at 302 nm due to the hydrolysis product, 2-mercapto-5-methylthiadiazole and there is also absorption at about 250 nm by unidentified degradation products. Ammonium vanadate [14], cerium (IV) [32] and molybdenum (VI) [16,20,22] have been used as oxidising agents in spectrophotometric determinations of β-lactam antibiotic. The excipients usually added in capsules, such as starch, glucose and lactose interfere in this cephalosporins determination [16]. 5,5′-Dithiobis(2-nitrobenzoic acid) (Ellman's reagent) [19] reacts with free thiol groups but a preliminary step of basic degradation is necessary. Cephalosporins, being carboxylic compounds, react with 2-nitrophenylhydrazine hydrochloride in presence of dicyclohexylcarbodi-imide and pyridine [24]. This method is general for carboxylic compounds just as penicillins and its degradation products. The method of the imidazole [25] is general for all cephalosporins containing an intact β-lactam ring fused to a six-membered ring with sulphur atom. Hence can be used as a stability indicating method and differentiates between the intact molecule and the degraded one. The acetylacetone–formaldehyde reagent method [26] is specific for amino β-lactam antibiotics. Non-amino β-lactam antibiotics and commonly encountered excipients did not interfere. But this method would not be stability indicating, because it cannot detect the cephalexin precursor, 7-amino-desacetoxy-cephalosporanic acid.

As can be seen in Table 1 the concentrations are generally at mg/l levels. Most of them require long reaction times, between 15 and 90 min for the ninhydrin and Ellman reagent, respectively, and temperatures from 30 to 100 °C.

Table 2 summarises some analytic properties of the HPLC methods [34–46]. These methods are

Table 1
Analytic properties of proposed methods for the analysis of cephalosporins by spectrophotometry UV–vis

Reagent	Drugs	Sample	λ (nm)	Reaction time (min)	pH	T (°C)	ε or slope of calibration	Interval dynamic	Limit detection	Consider-ations	Reference
Hydroxil-amine chloride	Cephalexin 7-ACA	Tablets, injection		Between 15–20		Ambient				Ni(II) as catalyst	[10]
Ninhydrin	Cefalotin		430	15		100	2.5×10^4 l/mol cm	3.2–32 μg/ml		Medium sulphuric acid	[11]
NaOH (hydrolysis)	Cephalexin		667	30			2.39×10^4 l/mol cm			Formation of methylene blue	[12]
	Cefuroxime			50			0.52×10^4 l/mol cm				
	7-ACA			40			1.76×10^4 l/mol cm				
NaOH (hydrolysis)	Cephalexin		667	Between 30–60		Boiling water bath	7.0×10^{-3} ml/μg	For all 8–80 μg/ml	0.3 μg/ml	Flow continous, automate, formation of methylene blue	[13]
	7-ACA						2.1×10^{-3} ml/μg		0.5 μg/ml		
	Cefuroxime						2.0×10^{-3} ml/μg		0.8 μg/ml		
	Cefazolin						7.0×10^{-3} ml/μg		0.6 μg/ml		
Ammonium vanadate	Cefalotin	Injection, capsules, tablets	750	10		Ebullition		20–100 μg/ml		Medium acid	[14]
	Cefaloridine			10							
	Cefapirine			10							
H_2SO_4 (hydrolysis)	Cefazolin	Commercial	302	20		Boiling water bath	1.3×10^4 l/mol cm	1–10 μg/ml		Medium sulphuric acid	[15]

Table 1 *(continued)*

Reagent	Drugs	Sample	λ (nm)	Reaction time (min)	pH	T (°C)	ε or slope of calibration	Interval dynamic	Limit detection	Consider-ations	Reference
Ammonium molibdate	Cefoxitin	Injections	684–696	30		Boiling water bath	7.1×10^3 l/mol cm	For all 20–100 μg/ml		Medium sulphuric acid	[16]
	Cefotaxime		686–697	30			5.9×10^3 l/mol cm				
Ninhydrin	Cefoxitin	Injection	458	5		Ambient	3.32×10^4 l/mol cm	2.5–15 μg/ml			[17]
NaOH (hydrolysis)	Cephalexin		266 (First derivative)	20	7.0			0.4–2.0 mg%		Deter. simultaneousas their products basic degradation first and second derivatives zero crossing	[18]
			273 (Second derivative)								
Ellman's reagent	Cefalotin	Pharmace	410	90	7.2	Boiling water bath		3.6–1.8 μg/ml	For all 2.4–6 μg/ml		[19]
	Cefacetril			65				3.2–1.6 μg/ml			
				90				5.0–25 μg/ml			
				90				6.0–30 μg/ml			
				90				2.4–18 μg/ml			
Phospho-molibdic acid	Cephalexin	Injections, capsules	700	25			2.9×10^3 l/mol cm			Medium Sulphuric acid	[20]
	Cefazolin			25			7.66×10^3 l/mol cm				
	Cefoxitin			25		98.5	9.05×10^3 l/mol cm				
	Cefotaxime			25			6.2×10^3 l/mol cm				

Table 1 *(continued)*

Reagent	Drugs	Sample	λ (nm)	Reaction time (min)	pH	T (°C)	ε or slope of calibration	Interval dynamic	Limit detection	Consider-ations	Reference
	Cefuroxime	Pharmaceuti	253 (First derivative)				5.30×10^{-4} ml/μg	Until 40 μg/ml	0.85 μg/ml (First derivative)	Deter. simultaneousas cefapirin first and second derivative zero crossing	[21]
			268 (Second derivative)				2.78×10^{-5} ml/μg		0.64 μg/ml (Second derivative)		
Phospho-molibdic acid	Cefuroxime		700	25		98.5	10.9×10^{3} l/mol cm				[22]
H_2SO_4 (hydrolysis)	Cefazolin	Capsules, vial, suspension	263	20		Boiling water bath		0.4–2.0 mg%		Deter. simultaneouss their products acid degradation second derivative	[23]
	Cephalexin		250/275	20							
	Cefotaxime		292	20							
2-Nitro-fenil hidrazine hydro-chloride	Cephalexin	Capsules, injections, suspension	537	15		60	2.87×10^{3} l/mol cm	For all 0.6–3 μmol/10 ml			[24]
	Cefotaxime			15			2.54×10^{3} l/mol cm				
	Cefazolin			15			2.62×10^{3} l/mol cm				
Imidazol	Cephalexin	Pharmaceuti	335	55	11.5	60		30–340 μg/ml			[25]
Acetil-ace-tona-phorm-aldehyde	Cephalexin	Tablets, capsules	400	30		30	2.68×10^{3} l/mol cm	10–100 μg/ml			[26]

Table 1 *(continued)*

Reagent	Drugs	Sample	λ (nm)	Reaction time (min)	pH	T (°C)	ε or slope of calibration	Interval dynamic	Limit detection	Consider-ations	Reference
NaOH (hydrolysis)	Cefotaxime	Capsules, suspension	670.1	30		Boiling water bath		0.5–7 μg/ml	0.05 μg/ml	Formation ethylene blue	[27]
Nitrate of cobalt NaOH	Cephalexin	Tablets	310	6		60					[28]
Cromotrope 2B	Cephalexin	Capsules	542	10	1.8	60		0.4–10 μg/ml			[29]
Cromotrope 2R			564	10	2.7	60		0.4–14 μg/ml			
Hydroxil-amine chloride	Cephalexin	Preparation pharmaceut	525	45		Ambient	40 μg/ml	80–320 μg/ml	510 μg/ml	Fe (III) as catalyst	[30]
	Cefotaxime						40 μg/ml		600 μg/ml		
Potassium sorbate	Cephalexin	Pharmaceut preparations	254 (First derivative)					5–100 μg/ml		First derivative absorbance	[31]
Ammonium Ce(IV) sulphate	Cefuroxime	Tablets	317	15–45		Boiling water bath		0.9–7.2 μg/ml		Medium sulphuric acid	[32]

frequently used for cephalosporin determinations in pharmaceutical samples in order to improve the selectivity. The UV detectors are generally used. The studied dynamic interval is in general at mg/l levels.

In this paper, we compare different methods proposed for the analysis of cefoxitin, cefotaxime, cephazolin and cephalexin. These cephalosporins have also been assayed spectrophotometrically after preliminary acid hydrolysis [15]. Acid degradation proceeds through cleavage of the side chain amide linkage [47]. The hydrolytic degradation of antibiotics is very often used as a preliminary step in the analytical procedures for their determination. The determination of cephalexin in pharmaceutical preparations is also studied.

On the other hand, the H-point Standard Additions Method (HPSAM) is a calibration method that transforms the uncorrectable error resulting from the presence of a direct interferent in the determination of an analyte into a constant systematic error. This error can then be evaluated and eliminated. This method also permits both proportional and constant errors produced by the matrix of the sample to be corrected directly. The basis of the method for known [48,49] or unknown [50,51] interferences were established.

We employed the calibration method HPSAM in order to test if the methods studied can be improved in their selectivity/specificity.

2. Experimental

2.1. Reagents

Stock solutions of sodium cefoxitin (Merck, Darmstadt), sodium cefotaxime (Hoescht Ibérica S.A.), sodium cephazolin (Lilly S.A., Alcobendas, Madrid, Spain) and cephalexin hydrate (Sigma, St. Louis, MO) of pharmaceutical grade were freshly prepared by dissolving 0.2000 g of the respective solid in 100 ml of distilled water (0.05 M sulphuric acid for cefotaxime). Stock-solutions of 7-ACA (Sigma) were prepared by dissolving 0.2500 g of the solid in 100 ml of water. The USP iodometric method provided the following contents for three replicates $106 \pm 7\%$ for cephazolin, $105 \pm 10\%$ for cefoxitin and $107 \pm 10\%$ for cefotaxime.

Working solutions were prepared by dilution as required. Sulphuric acid 96% (Panreac, Barcelona, Spain), sodium hydroxide (Probus, Barcelona, Spain), sodium thiosulphate (Probus), potassium iodide (Scharlau, Barcelona, Spain) and iodine (D'Hemio, Madrid, Spain) were also used. All solutions were made in distilled water and all reagents used were analytical-grade chemicals.

For the application of HPLC method, standard solutions of sodium cefoxitin, sodium cephazolin, cephalexin hydrate and sodium cefuroxime (Glaxo, Aranda of Duero, Spain) of pharmaceutical grade were prepared by dissolving 0.0158, 0.0158, 0.0161, 0.0105 g of the respective solid in 10 ml of distilled water. The stock solution of sodium cefotaxime was prepared by dissolving 0.0263 g in 25 ml of distilled water. Working solutions were prepared by dilution as required.

Acetonitrile was HPLC grade from Scharlau. Water was distilled, deionized and filtered through 0.45-mm nylon membranes (Tecknokroma, Barcelona, Spain). All the samples were filtered before their injection in the analytic column with nylon filters (0.45 mm, Tecknokroma).

The NaH_2PO_4 solution was prepared by dissolving 3.5 g of sodium dihydrogen phosphate (Probus) in 500 ml of distilled water. The pH was adjusted to 3 by adding 50% H_3PO_4.

2.2. Mobile phase

A gradient of acetonitrile: NaH_2PO_4 (5×10^{-2} M, pH 3) with and increasing acetonitrile content from 10% v/v at zero time, 20% v/v at 2 min, 20% v/v at 6 min and 50% v/v at 8 min was used. The solution was prepared daily, filtered through a 0.45-mm nylon membrane (Tecknokroma) and degassed with helium before use. The flow rate was 0.75 ml/min. For quantitative determination a chromatographic signal was monitored at 254 nm. Nine replicates were made.

2.3. Dosage forms

They were obtained from local sources; several formulations were used.

Kefloridina forte, 500 mg of cephalexin monohydrate for capsule (Lilly S.A.).

Kefloridina suspension, 250 mg of cephalexin monohydrate for packet (Lilly S.A.).

Kefloridina mucolitico, 500 mg of cephalexin monohydrate and 8 mg of bromhexine chlorhydrate for capsule (Lilly S.A.).

Kefloridina mucolitico suspension, 250 mg of cephalexin monohydrate and 4 mg of bromhexine chlorhydrate for packet (Lilly S.A.).

All solutions were made in water and all reagents were analytical-grade chemicals.

2.4. Equipment

Spectrophotometric measurements were made in a Perkin-Elmer Lambda 16 UV–vis double-beam equipped with 1-cm pathlength quartz cells and interfaced to an Ataio S-2000 AT computer and an Epson LQ-400 printer. The spectra were recorded between 350 and 550 nm at 1 nm intervals.

A Hewlett-Packard HP 8452A diode-array spectrophotometer equipped with a 1-cm pathlength quartz cell and interfaced to a HP Vectra ES/12 computer and HP Think-Jet printer was also used. The spectra were recorded between 350 and 550 nm at 2 nm intervals.

pH measurements were made with a Crison micropH 2000 pH-meter (Crison Instruments, CORP., Alella, Barcelona, Spain).

A Hewlett-Packard 1040A liquid chromatograph, equipped with a diode-array detector (Hewlett-Packard, 1040 Series), linked to a data system (Hewlett-Packard HPLC Chem Station, Palo Alto, CA) was used for data acquisition and storage. The system was coupled to a quaternary pump (Hewlett-Packard, 1050 Series) and an automatic sample injector (Hewlett-Packard, 1050 Series). The column was a Hypersil ODS-C18 5 m (125×4 mm^2 i.d.) (Hewlet-Packard, Darmstadt, Germany). The detector was set to collect a spectrum every 640 ms (over the range of 220–600 nm) and all the assays were carried out at room temperature.

To calculate all statistical least squared calibration lines at all the measured wavelengths from the spectral data and the calculations of the HPSAM, a standard computer program package (StatView 4.02 from ABACUS Inc.) for the Macintosh and the Excel of Microsoft Office 97.

3. Procedures

These procedures were prepared according to [52,53].

3.1. Standard solutions of cepahlosporins

3.1.1. Assay of intact cephalosporins

Different volumes between 0.2 and 1.6 ml of stock solutions of cephazolin and cefoxitin, and between 0.4 and 1.6 ml for the cefotaxime were diluted with distilled water (or acid sulphuric 0.05 mol/l for the cefotaxime) to 100 ml. Different volumes between 0.1 and 1.6 ml of stock solutions of cephalexin and between 0.2 and 1.6 ml of 7-ACA were diluted with water to 50 and 10 ml, respectively. The absorbance between 210 and 400 nm was recorded against distilled water.

3.1.2. Assay of degraded cephalosporins

These products are prepare as in Ref. [23]: 20 mg cephazolin, cefoxitin, cefotaxime and cephalexin, 25 mg of 7-ACA or 10 ml of water for the preparation of the blank, were transferred into 100 ml volumetric flasks using 10 ml of 4.5 mol/l sulphuric acid. The solution was heated in a boiling water bath for 20 min, then it was cooled and neutralised with 6.0 mol/l sodium hydroxide solution and made up the volume to the mark with water (or sulphuric acid 0.05 mol/l for cefotaxime). Different volumes between 0.4 and 1.8 ml of stock solutions of cephazolin, cefoxitin, cefotaxime were diluted appropriately to 50 ml. Different volumes between 0.2 and 1.6 ml of the stock solutions of cephalexin and 7-ACA, respectively, or 5 ml of the blank solution were diluted to 25 ml. The absorbance was registered as mentioned above.

3.1.3. Assay of cefoxitin with ninhydrin [54]

The procedure used was that of Mahrous and Abdel-Khalek [17]: different volumes of the stock solutions of cefoxitin (4 mg of solid in 25 ml) between 0.10 and 1.00 ml and up to 1.00 ml of distilled water is added (if required), were transferred into a 10 ml calibrated flask. A 0.10-ml volume of 1% ninhydrin solution followed by 2.00 ml of concentrated sulphuric acid were added. The solution was shaken gently and was left standing for 5 min at room temperature. The contents were diluted to 10 ml with 50% sulphuric acid. After 5 min, the absorbance between 300 and 600 nm was recorded against distilled water.

3.1.4. Assay of degraded cefoxitin with ninhydrin

The procedure used is that was described in Ref. [23]: 200 mg of cefoxitin was transferred into a 100 ml calibrated flask using 10 ml of 9 M sulphuric acid. The solution was heated in a boiling water bath for 20 min, then cooled and neutralised with a 9 M sodium hydroxide solution. The volume was filled up to the mark with water. 10 ml of this stock solution was diluted to 50 ml. Different volumes of this solution were transferred into a 10 ml calibrated flask, and the subsequent experimental procedure followed the above guidelines for calibration graphs of cefoxitin. The absorbance was registered as mentioned above.

3.2. Applications to pharmaceutical samples [53]

3.2.1. Preparation of sample

3.2.1.1. Intramuscular injections. The stock solutions of each cephalosporin were prepared dissolving the appropriate quantities of the powder in 100 ml of distilled water.

3.2.1.2. Capsules. Thoroughly mix the contents of 5 capsules, weigh, transfer an accurately weighed quantity of powder equivalent to 183 mg of cephalexin to a 100 ml, volumetric flask, and dissolve in and dilute to volume with water. Shake well and filter through Whatman No. 42 paper. Discard the first portion of filtrate. Use the clear solutions obtained as stock solutions (0.005 mol/l).

3.2.1.3. Oral suspension. Accurately weigh a quantity of powder equivalent to 183-mg cephalexin, and treat as described under capsules.

3.2.2. Degradation of samples

3.2.2.1. Intramuscular injections. As it is indicated in Section 3.1.2 [44].

3.2.2.2. Capsules and oral suspension. Accurately weigh a quantity of powder equivalent to 286-mg cephalexin, and prepare a solution as described above. Add to 10 ml of this solution 10 ml of 9.0 mol/l sulphuric acid, and treat the solution as described for the degraded cephalosporins [23].

3.3. Method of the ninhydrin

The products of reaction of the cefoxitin with ninhydrin were prepared as indicated in Section 3.1.3.

The products of the reaction of the cefoxitin degraded with ninhydrin were prepared as indicated in Section 3.1.4.

3.4. High-performance liquid chromatography

The pharmaceuticals samples of the cephalexin were prepared as indicated above. The solutions of Kefloridina forte capsules, Kefloridina oral suspension, Kefloridina forte mucolitico capsules and Kefloridina suspension oral mucolitico were prepared dissolving 0.0030, 0.0333, 0.0034 and 0.0333 g, respectively, in 25 ml of distilled water.

4. Results and discussion

The precision of different methods used for the determination of cefoxitin, cephazolin, cefotaxime and cephalexin alone and in the presence of bromhexine is evaluated. Fig. 2 shows the percentage recovery $\pm$ standard deviation calculated for cefoxitin when different methods are employed. The wavelengths utilised for intact cefoxitin are: 240 nm for absorbance; 244 and 280 nm for first derivative absorbance; 236, 252, 270 and 292 nm for second derivative absorbance; and for

HPSAM the pairs of λ are 240–276, 224–280 and 250–272 nm. For acid degraded cefoxitin the wavelengths utilised are 240 nm for absorbance, 248 nm for first derivative absorbance, and 240, 256 and 270 nm for second derivative absorbance. Using ninhydrin method, the wavelengths are 454 nm for absorbance, and 424 and 474 nm for first derivative absorbance. According to fundamentals of the HPSAM applied to analytical procedures that use a reagent blank, two wavelengths are selected in which the external and internal blanks show the same absorbance [55,56]. The wavelengths selected are 456–476 nm and 444–492 nm. The results of HPLC method are obtained at 254 nm.

Good precision is obtained working with the form intact. The worst results are obtained working with the degraded form and using the second derivative.

In a previous paper [54], we shown that different products were obtained from cefoxitin with and without hydrolysis by sulphuric acid and reaction with ninhydrin. An in situ calibration is read in order to prevent the possible formation of acid degraded cefoxitin–ninhydrin product when it is working with the intac antibiotic. Because of this when using the ninhydrin method the best results are obtained applying the HPSAM method. It may be that the influence of the acid degraded cefoxitin–ninhydrin compound has been eliminated. This influence is more important when the measured cefoxitin concentration is smaller. The precision obtained by the HPSAM/ninhydrin is similar to those obtained using HPLC method and iodometric method.

Table 3 gives the relative recovery for all methods tested for intact and degraded cephazolin. The worst precision corresponds to iodometric method and second derivative using Lambda 16 spectrophotometer. Good results are obtained with the others methods.

As can be seen in Table 4 for intact and degraded cefotaxime, the worst precision is obtained using iodometric method. The others results obtained are suitable. From Table 5 for cephalexin, similar conclusion can be derived.

The precision obtained when these methods are applied to the determination of cephalexin in different pharmaceutical samples is similar to that obtained for cephalexin standard. In a previous

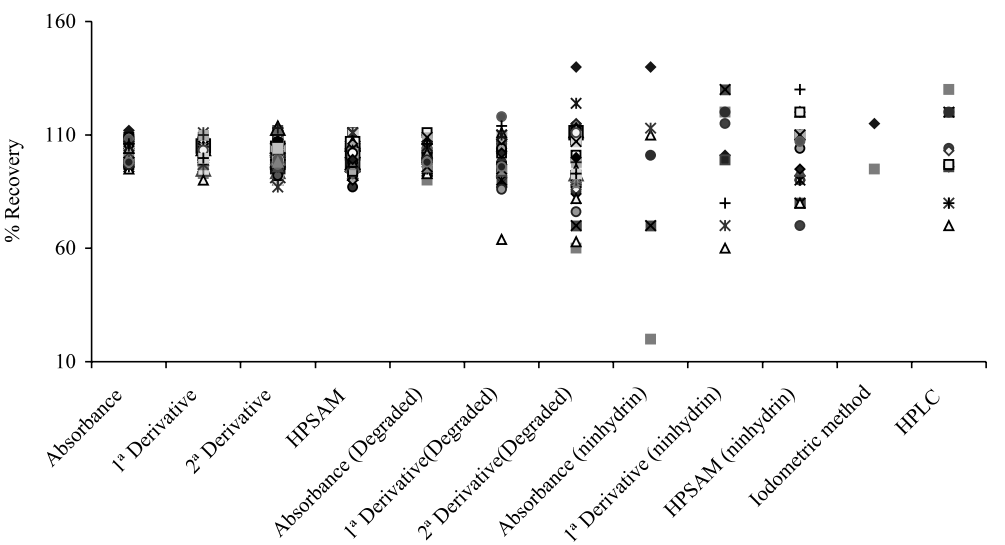


Fig. 2. Recovery percentage ± standard deviation found in the analysis of cefoxitin using different methods; number of replicates is three for each result. Range concentrations: $(1.04–8.19) \times 10^{-5}$ mol/l for intact, and acid degraded, $(0.36–3.56) \times 10^{-5}$ mol/l using ninhydrin method and $(0.06–117) \times 10^{-5}$ mol/l for HPLC. 4.45×10^{-3} mol/l for iodometric method. The various symbols (◆–△) show different concentrations included in the range studied.

Table 2
Analytic properties of the proposed methods for the analysis of cephalosporins for HPLC

Drugs	Sample	Phase stationary, phase mobile	Detection λ (nm)	Interval dynamic concentrations	Limit detection	Considerations	Reference
Cefoxitin	Injection	Zipax SAX (resin interchange ionic) acetic acid–water pH 5	UV 254		Standard 2–500 μg/ml	HPLC of interchange anionic	[34]
Cephalexin		μBondapak C_{18} acetonitrile–buffer phosphate pH 5.5	UV 325 fluorescence 350/450	26 μg/ml	0.5 μg/ml	Pre-column react. imidazole-metal salt post-column as *o*-phthaldialdehyde HPLC phase reverse	[35]
7-ACA cephalexin		Zorbax™ C_8 5 μm sodium perchlorate pH 2.5	UV 254			Separation of epimers varying the phase mobile HPLC in phase reverse	[36]
Cefazolin	Ointment ophthalmic	μBondapak C_{18} (10μm) buffer ammonium acetate pH 6.8 methanol	UV 254	5–40 μg/ml	0.18 mg/g	HPLC phase reverse	[37]
Cefazolin		5 μm ODS C_{18}, A: water–methanol–acetic acid, B: ammonium acetate pH 5	UV 262 ME	0.05–0.5 μg/ml	1 ng	HPLC-mass spectrometry (ME)	[38]
Cephalexin		YMC-ODS 5 μm Na_3PO_4 pH 4-acetonitrile	UV (220) ME			Isolation and determination impurity antibiotics	[39]
7-ACA		Spheri 5 ODS-224	UV 260			Study: retention of cefalosp. in MLC influence: pH, conc., surfactants, modifier	[40]
Cefazolin		Sodium sulphate dodecil					
Cephalexin							
Cephalexin	Granules, capsules, powders	μBondapak C_{18} (10 μm), Methanol–acetic acid glacial	UV 254	0.2–1.75 mg/ml		HPLC phase reverse	[41]
Cefuroxime			UV (278) fluorescence (462/500)		0.13 μg/ml	Derivation as 5-bromometil fluoresceine	[42]

Table 2 *(continued)*

Drugs	Sample	Phase stationary, phase mobile	Detection λ (nm)	Interval dynamic concentrations	Limit detection	Considerations	Reference
Carboxilic acids			Argon laser induced fluorescence 458				
Cephalexin Cefoxitin	Injection	5 μm Hypersil ODS C18 KH_2PO_4 buffer pH 3.4 acetonitrile	UV 254	0.099–16.4 μg			[43]
Cefotaxime	Injection	10 μm YWG-C18 acetate buffer pH 5/methanol/acetonitrile	UV 254	4.9–99 μg/ml	2.4 ng		[44]
Cephalexin	Capsules	10 μm YWG-C18 acetate buffer pH 5/methanol/acetonitrile	UV 254	18–535 μg/ml		Study of impurity 7-ACA, phenylglycine	[45]
Cephalexin	Capsules	Spherisorb 3 μm CN 60% methanol in water	UV 214			Simultaneous determ. bromhexin, cephalexin	[46]

MLC: micellar liquid chromatography.

Table 3
Relative recovery of cephazolin by applying the calibrates (a) of intact standards, HPLC and iodometric method (b) of acid degraded standards

Measured concentration $M \times 10^{-5}$	Relative recovery[a] $\pm s$												
	Iodometric method	Absorbance		Second derivative				HPSAM						HPLC ($\lambda = 254$ nm)
		$\lambda = 272$ nm		$\lambda = 280$ nm		$\lambda = 302$ nm		240–276		224–280		250–270		
		(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	
0.84		101 ± 6		88 ± 11				106 ± 7		–		102 ± 7		
1.68		102 ± 4	100.5 ± 1.6	95 ± 8	100.4 ± 1.4	97 ± 11	99.6 ± 1.3	103 ± 8	98.4 ± 1.9	–	109 ± 3	101 ± 7	99.2 ± 1.9	
2.52		102 ± 2	99.9 ± 0.5	102 ± 6	100.6 ± 0.4	102 ± 8	100.3 ± 0.4	104 ± 2	100.7 ± 1.0	103 ± 6	110.2 ± 1.3	102 ± 1	101.1 ± 1.1	
3.36		102.9 ± 1.8	99.9 ± 0.3	102 ± 6	100.2 ± 0.2	104 ± 7	100.3 ± 0.3	105.6 ± 0.7	101 ± 1	107 ± 4	109.4 ± 1.2	104.3 ± 0.3	101.1 ± 0.8	
4.20		98.7 ± 1.6	99.9 ± 0.4	96 ± 3	99.77 ± 0.18	95 ± 5	100.00 ± 0.15	99.7 ± 1.6	100.6 ± 0.4	99 ± 5	107 ± 2	99.2 ± 1.3	100.7 ± 0.4	
5.04		100 ± 2	99.8 ± 0.4	97 ± 3	99.3 ± 0.3	98 ± 4	99.6 ± 0.3	100.8 ± 1.9	99.8 ± 0.8	101 ± 4	103.0 ± 0.9	100.2 ± 1.5	99.9 ± 0.9	
5.51														101 ± 1
5.88		100.2 ± 1.4	100.2 ± 0.3	98 ± 3	99.8 ± 0.2	97 ± 4	100.1 ± 0.2	100.6 ± 1.7	100.3 ± 0.7	101 ± 2	103.6 ± 0.9	100.1 ± 1.8	100.5 ± 0.7	
6.72		102 ± 3	100.1 ± 0.4	99 ± 3	100.2 ± 0.4	99 ± 3	100.2 ± 0.4	102.1 ± 1.7	100.3 ± 0.8	102 ± 3	103.8 ± 0.6	101.6 ± 1.6	100.4 ± 0.9	
7.56			100.0 ± 0.8		100.3 ± 0.8		99.9 ± 0.6		100.0 ± 0.4		102.9 ± 0.4		100.1 ± 0.4	
22.05														98 ± 4
44.10														100.0 ± 1.8
66.16														100.4 ± 0.6
88.21														100.6 ± 1.3
110.26														99.6 ± 0.7
421.42	102 ± 17													

Measured concentration $M \times 10^{5}$	Relative recovery[b] $\pm$ s							
	Absorbance		Second derivative					
	$\lambda = 288$ nm		$\lambda = 260$ nm		$\lambda = 288$ nm		$\lambda = 314$ nm	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
0.84	102 ± 8		97 ± 3		98 ± 13		97 ± 7	
1.68	96 ± 2	101.96 ± 3	100 ± 5	100 ± 3	96 ± 7	101 ± 2	94 ± 4	97.0 ± 1.5
2.52	98 ± 2	99 ± 1	101 ± 3	99.2 ± 1.4	99 ± 4	99.6 ± 0.8	102 ± 4	99 ± 1
3.36	98.8 ± 1.8	100.8 ± 0.9	102.8 ± 1.5	10.7 ± 0.6	100 ± 4	100.1 ± 0.4	102 ± 3	101.1 ± 1.7
4.20	97.0 ± 1.5	99.3 ± 0.7	101 ± 2	99.8 ± 0.5	98 ± 3	99.5 ± 0.6	94 ± 7	101.0 ± 0.8
5.04	101 ± 2	99.2 ± 0.4	98.1 ± 1.8	99.8 ± 0.6	99 ± 2	99.64 ± 0.17	98.3 ± 1.3	100 ± 2
5.88	101.0 ± 1.6	101.0 ± 0.8	100 ± 4	100.8 ± 0.6	100.4 ± 0.5	100.5 ± 0.6	100.6 ± 1.7	101.6 ± 0.7
6.72	100.4 ± 1.1	100.1 ± 0.4	100.7 ± 1.5	99.2 ± 0.5	98.4 ± 0.6	99.9 ± 0.3	99.0 ± 0.8	99.1 ± 0.7
7.56		99.7 ± 0.3		100.2 ± 0.3		100.0 ± 0.4		99.5 ± 0.4

(1) Measures made with the Lambda 16 spectrophotometer.(2) Measures made with the spectrophotometer of diodes.

Table 4
Relative recovery of cefotaxime by applying the calibrate: of intact and acid degraded standards, HPLC and iodometric method

Measured concentration $M \times 10^5$	Relative recovery $\pm s$												
	Iodometric method	Intact								Degraded				HPLC
		Absorbance	Second derivative				HPSAM			Absorbance	Second derivative			
		262 nm	242 nm	260 nm	288 nm	310 nm	224–280	234–278	240–276	246 nm	260 nm	276 nm	308 nm	254 nm
1.68		100 ± 6	99 ± 6	98 ± 6	101 ± 5	100 ± 7	102 ± 5	103 ± 5	104 ± 5	100 ± 6	99 ± 6	100 ± 5	100 ± 10	
2.51		101 ± 2	100.8 ± 1.8	100.5 ± 1.7	101.2 ± 1.6	101 ± 3	103 ± 3	104 ± 3	105 ± 3	102 ± 5	99 ± 6	100 ± 6	99 ± 6	
3.35		99.4 ± 0.8	99.5 ± 0.7	99.6 ± 0.7	99.3 ± 0.7	99.4 ± 1.3	102.6 ± 0.7	102.8 ± 0.9	103.9 ± 1.1	100 ± 6	100 ± 5	99 ± 3	100 ± 5	
4.19		100 ± 1	100.1 ± 0.9	100.3 ± 0.8	99.2 ± 0.8	99.5 ± 0.6	101 ± 2	102 ± 2	102 ± 2	99 ± 4	100 ± 3	100 ± 3	100 ± 3	
5.03		100.2 ± 0.5	100.7 ± 0.6	100.9 ± 0.4	99.6 ± 0.4	100.1 ± 0.2	102.2 ± 1.7	102.6 ± 1.6	103.4 ± 1.8	98 ± 3	99.9 ± 1.9	100 ± 2	99 ± 2	
5.87		99.9 ± 0.8	100.1 ± 0.8	100.4 ± 0.9	99.9 ± 0.8	99.9 ± 1.1	102.7 ± 1.6	102.9 ± 1.9	103.6 ± 2	99 ± 6	100 ± 5	100 ± 3	99 ± 5	
6.70		100.4 ± 0.6	100.0 ± 0.5	100.25 ± 0.18	100.3 ± 0.3	100.2 ± 0.2	101 ± 2	102 ± 2	102 ± 2	105 ± 5	104 ± 5	103 ± 3	105 ± 5	
7.54		99.8 ± 0.7	99.7 ± 0.8	99.2 ± 0.7	100.2 ± 1.1	100.0 ± 0.7	101 ± 2	101.0 ± 1.3	101.3 ± 1.0	98 ± 4	96.9 ± 1.8	98.3 ± 1.7	98 ± 2	
22.0														99 ± 3
44.10														103 ± 4
66.00														100.7 ± 0.5
88.21														99.1 ± 1.0
110.26														99.8 ± 1.2
418.90	112 ± 18													

Table 5
Relative recovery of cephalexin by applying the calibrates: of intact and acid degraded standards

Measured concentration $M \times 10^{-5}$	Relative recovery $\pm s$													
	Intact					Degraded								
	Absorbance	First derivative		Second derivative		Absorbance	First derivative				Second derivative			
	262 nm	250 nm	278 nm	266 nm	286 nm	348 nm	260 nm	280 nm	324 nm	370 nm	272 nm	290 nm	348 nm	386 nm
0.57						118 ± 36				104 ± 40				
1.14	138 ± 7	–	128 ± 11	117 ± 11	127 ± 12	102 ± 17	–	102.2 ± 1.3		101 ± 5	80 ± 30	109 ± 9	97 ± 9	100 ± 10
1.70						105 ± 12		91 ± 13	93 ± 17	110 ± 15		81 ± 40	80 ± 30	90 ± 30
2.27	100 ± 10	101 ± 5	101 ± 7	101 ± 5	104 ± 6	102 ± 7	92 ± 8	95 ± 8	100 ± 10	104 ± 9	102 ± 16	95 ± 6	95 ± 4	95 ± 3
2.83						106 ± 7	99 ± 8	100 ± 7	100 ± 10	106 ± 8	109 ± 9	101 ± 6	100 ± 6	100 ± 5
3.41	110 ± 3	106 ± 5	108 ± 4	105 ± 4	108 ± 4	97 ± 7	94 ± 5	97 ± 6	97 ± 8	103 ± 7	93 ± 6	95 ± 4	97 ± 3	96 ± 2
3.96						102 ± 5	80 ± 20	100 ± 6	99 ± 7	104 ± 6	130 ± 30	96 ± 13	96 ± 11	100 ± 12
4.54	100 ± 7	100 ± 4	100 ± 6	99 ± 4	100 ± 5	100 ± 5	94 ± 5	98 ± 6	99 ± 6	101 ± 7	90 ± 10	95 ± 5	97 ± 4	95 ± 5
5.68	106.8 ± 1.6	104 ± 3	105 ± 2	104 ± 2	104 ± 2	104 ± 4	92 ± 9	103 ± 5	102 ± 4	104 ± 4	102 ± 4	101 ± 8	100 ± 6	101 ± 7
6.82	99 ± 5	100 ± 5	100 ± 5	100 ± 5	99 ± 4	102 ± 3	94 ± 5	102 ± 4	102 ± 4	102 ± 4	108 ± 17	100 ± 6	100 ± 5	101 ± 6
7.95	101.8 ± 1.2	100.9 ± 1.6	100.9 ± 1.4	101.5 ± 1.6	100.8 ± 1.5	103 ± 3	97 ± 3	102 ± 4	102 ± 3	102 ± 3	106 ± 14	101 ± 5	100 ± 4	101 ± 4
8.48						102.5 ± 1.8	100 ± 2	103 ± 4	103 ± 3	103 ± 3	104 ± 12	102 ± 4	101 ± 3	102 ± 4
9.09	100 ± 4	101 ± 4	100 ± 4	101 ± 4	100 ± 4	99 ± 3	95 ± 4	97 ± 6	98 ± 4	98 ± 4	90 ± 7	97 ± 5	98 ± 4	97 ± 5
11.4	98 ± 5	99 ± 5	97 ± 5	99 ± 5	98 ± 5	103.0 ± 1.7	104 ± 4	104 ± 4	104 ± 3	103 ± 2	111 ± 12	104 ± 4	103 ± 2	103 ± 3
13.6	97 ± 2	97 ± 2	96.5 ± 1.7	97 ± 2	97.1 ± 1.9	99.7 ± 1.8	98 ± 5	97 ± 4	98 ± 3	98.5 ± 1.9	93 ± 9	97 ± 4	98 ± 2	97 ± 3
14.1						103.1 ± 1.6	106 ± 6	104 ± 5	104 ± 3	103.7 ± 1.7	105 ± 7	105 ± 5	103 ± 2	104 ± 3
15.9	101.1 ± 1.6	100.4 ± 1.9	101 ± 2	100.3 ± 1.9	101.1 ± 1.7									
17.0						102.0 ± 1.6	106 ± 8	103 ± 5	103 ± 3	102.4 ± 1.4	103 ± 4	104 ± 5	103 ± 2	103 ± 4
18.2	100.1 ± 0.5	99.2 ± 1.0	99.8 ± 0.2	99 ± 1.4	100.3 ± 0.4	97.8 ± 1.7	97 ± 6	94 ± 4	95 ± 3	97.1 ± 1.5	83 ± 5	94 ± 4	96.4 ± 1.8	96 ± 2
19.8						101.9 ± 1.6	106 ± 9	103 ± 5	102 ± 3	102.3 ± 1.2	104 ± 3	103 ± 5	103 ± 3	103 ± 4
22.6						99.9 ± 1.7	100 ± 8	98 ± 5	99 ± 3	99.9 ± 1.6	93 ± 6	99 ± 5	100 ± 3	99 ± 3
27.1						98.6 ± 1.6	94 ± 8	95 ± 4	98 ± 2	98.2 ± 1.2	93 ± 3	95 ± 4	97.7 ± 1.5	97 ± 2
31.6						98.9 ± 1.6	90 ± 8	94 ± 4	96 ± 2	98.7 ± 1.2	108 ± 7	95 ± 3	97.9 ± 1.5	97 ± 2
36.2						100.0 ± 1.6	81 ± 7	94 ± 4	95 ± 2	99.3 ± 0.9	94 ± 7	89 ± 7	92 ± 7	91 ± 7

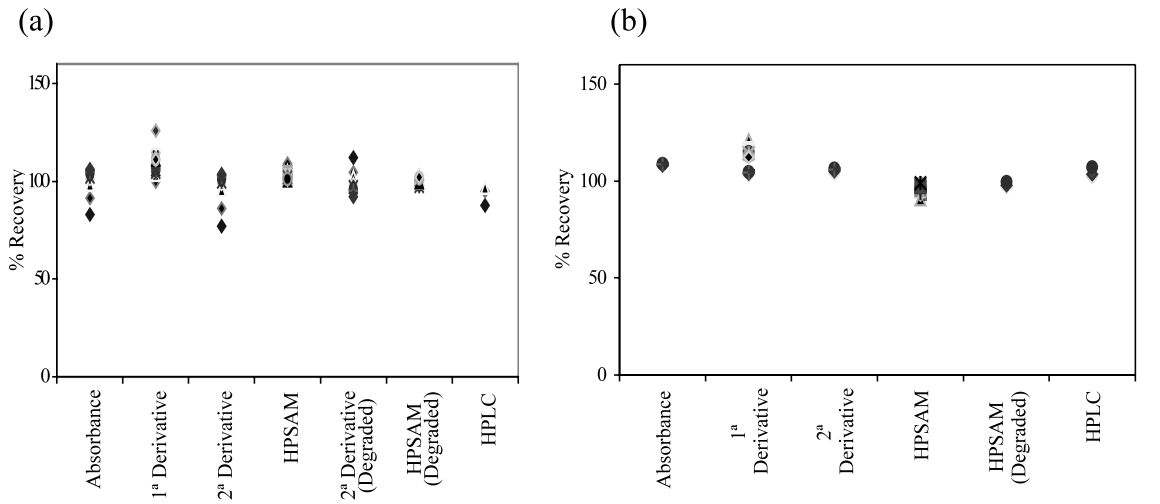


Fig. 3. Average recovery percentage found for three replicates of each result using different methods of determination of cephalexin in capsule. The range of concentrations studied were: $(1.06–27.95) \times 10^{-5}$ mol/l and $(2.03–36.56) \times 10^{-5}$ mol/l for cephalexin alone (a) and in presence of bromhexine (b) respectively. The various symbols (◆–△) show different concentrations included in the range studied.

paper [52], we found that the excipients usually added in pharmaceutical samples, such as starch, talc, dicalcium phosphate and lactose did not disturb these determinations. Fig. 3 and Fig. 4 show the average recovery percent obtained when using different methods for the analysis of pharmaceutical samples of cephalexin. When HPSAM is applied to the determination of intact cephalexin the pairs of λ are 224–280, 208–230 and 212–280 nm. Whereas, for the degraded are 230–288 and 224–276 nm.

The wavelengths utilised when the others methods are applied are shown in Table 5 for intact and acid degraded standards.

As can be seen in Fig. 3 and Fig. 4 good results are obtained using all the methods for the determination of intact and degraded cephalexin alone and in presence of bromhexine. The best accuracy is obtained using HPSAM.

5. Conclusions

This paper shows several methods for the determination of intact and degraded cephalosporins. It is demonstrated that the intact cephalosporins determination provides, generally, better results than those estimated by acid degraded compound determination. But the direct spectrophotometric methods suffer a lack of specificity because all compounds containing the β-lactam ring absorb in the range 250–270 nm. Besides, the latter methods can improve selectivity because the impurity 7-ACA that can be present in the trade product also shows less interference. Also, acid degraded cephalosporins spectra present more significance difference than intact drugs spectra.

When using the ninhydrin method, the application of the HPSAM reduces the blank bias error and the interference of other species. HPSAM permits the determination of cefoxitin in presence of degraded cefoxitin. 7-ACA does not bear the 2-thienyl moiety and failed to condense with ninhydrin under the conditions of determination of cefoxitin. HPSAM also provides a precision similar to HPLC and iodometric methods for this cephalosporin.

For cephazolin, cefotaxime and cephalexin the precision achieved by intact or acid degraded UV–vis spectrophotometry and HPLC was good and similar. Worse precision was obtained by iodometric method.

Good results are obtained in the analysis of intact cephalexin in all pharmaceutical samples.

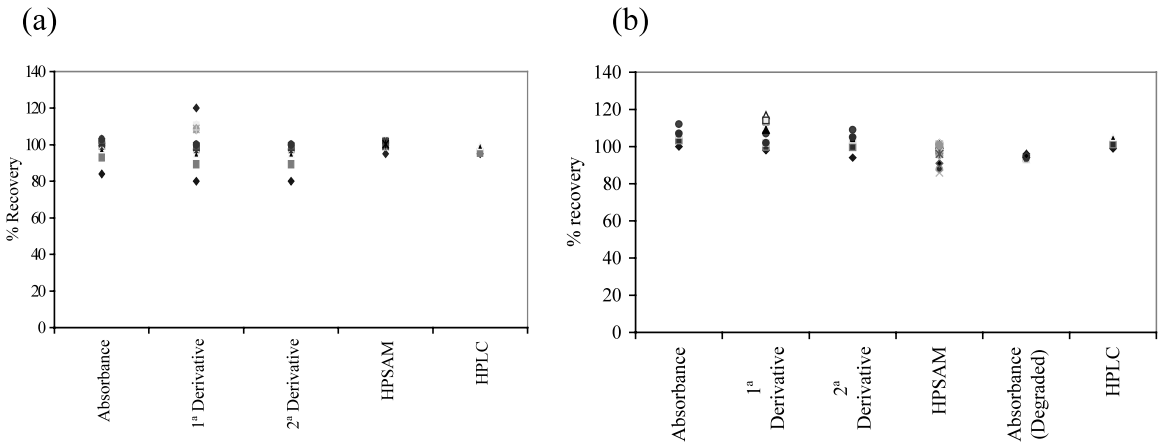


Fig. 4. Average recovery percentage found for three replicates of each result using different methods of determination of cephalexin in suspension. The range of concentrations studied were: $(1.06–28.83) \times 10^{-5}$ mol/l and $(2.163–30.49) \times 10^{-5}$ mol/l for cephalexin alone (a) and in presence of bromhexine (b) respectively. The various symbols (◆–△) show different concentrations included in the range studied.

However, working with degraded cephalexin the calibration method should be optimised. HPSAM is the best options for pharmaceuticals tested.

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