Simultaneous determination of paracetamol and cephalexin binary mixtures by using derivative spectrophotometry and H-point standard addition methods

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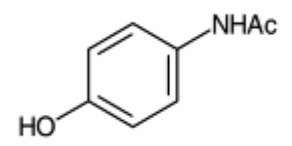
Abstract

Two methods are applied for simultaneous determination of paracetamol (para) and cephalexin (ceph) in combined mixtures, derivative spectrophotometric technique and H-Point standard addition method (HPSAM) spectrophotometry. The first procedure is based on the use of the, second derivative (^{٢}D) and used the suitable wave lengths at zero crossing at valley ٢٥٠,٥ and ٢٩٠,٢ nm for determination paracetamol and cephalexin respectively.. Calibration graphs were established for ٢-٦٠ mg/L for Para and ٢-٧٠ mg/L for ceph in binary mixture. The proposed method were successfully applied to the determination of these drugs in synthetic mixtures, linearity was excellent ($r^{٢}$>٠,٩٩٩) over the concentration tested (٢–٦٠ mg/L) with good precision and accuracy. Recoveries were good (>٩٨٪) with limits of detection of ١ and ١,٥ mg/L ,(%R.S.D.= ٠,٧٥٧- ١,٢٠٧) for para and ceph, respectively.. The second procedure HPSAM was applied to the simultaneous determination of Para and ceph. respectively. (HPSAM) method was based on the difference in the absorbance of para and ceph.at choosing wavelengths (٢٦٥ and ٢٥٧ nm). Results of the analysis of the different mixtures in the proposed system revealed a dynamic range of ٢-٣٥ and ٢-٥٠ mg/L, with limits of detection of ٢ and ٣ mg/L for para and ceph, respectively. The relative error are between -٠,٦٠٥-١,٦٤ and -١,٢٣٠-١,٤٣, for para and ceph respectively. RSD% ٠,١٥١-١,٧٧٥ for para

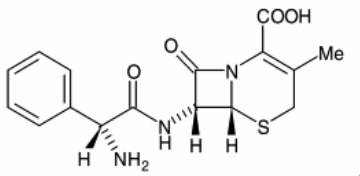
and ceph, respectively. The two methods were found to be precise, accurate, simple and rapid..

Introduction:

Paracetemol is acetaminophen is the active metabolite of phenacetin, a so-called coal tar analgesic. Unlike phenacetin, paracetamol has not been shown to be carcinogenic in any way. It has analgesic and antipyretic properties, but, unlike aspirin, it is not a very effective anti-inflammatory agent. It is well tolerated, lacks many of the side-effects of aspirin, and is available over-the-counter, so it is commonly used for the relief of fever, headaches, and other minor aches and pains. Paracetamol is also useful in the management of more severe pain, where it allows lower dosages of additional non-steroidal anti-inflammatory drugs (NSAIDs) or opioid analgesics to be used, thereby minimizing overall side-effects. It is a major ingredient in numerous cold and flu medications, including Tylenol and Panadol, among others. It is considered safe for human use at recommended doses; however, acute overdose can cause fatal liver damage often heightened with use of alcohol, and the number of accidental self-poisonings and suicides has grown in recent years[1]. The words *acetaminophen* and *paracetamol* come from the chemical names for the compound: *para*-acetylaminophenol and *para*-acetylaminophenol. (The brand name Tylenol also derives from this name: *para*-acetylaminophenol.) In some contexts, it is shortened to APAP, for *N*-acetyl-para-aminophenol.[١] It has the formula $C_8H_9NO_2$, molecular weigh ١٥١,٢ and structure as follow.



Paracetemol



cephalexin

Paracetemol is a white, crystalline powder, sparingly soluble in water (٠,١-٠,٥ g/١٠٠mL at ٢٠ C^{o}) , freely soluble in alcohol, very slightly soluble in ether and in methylene chloride[٢]. Paracetamol (acetaminophen) is widely used as an analgesic and as an antipyretic drug. Many assays have been described for acetaminophen including titrimetry [٣], chromatography [٤-٩], fluorometry [١٠], colorimetry [١١-١٤], UV spectrophotometry [١٥], and various modes of electrochemistry [١٦-٢٤]. Although the electrochemical oxidation of paracetamol at a glassy carbon electrode has been in the literature for some time[٢٠] only a few applications of its use in differential pulse voltammetry have been reported; for determination of the drug in blood plasma and in a single type of tablet [٢١] and in a variety of drug formulations containing paracetamol [١٩]. Recently the differential pulse voltammetric behaviour of some drugs including paracetamol at various conducting polymers [١٧] and at pumice mixed carbon electrodes [٢٢] have been examined and reviewed [٢٣]. cyclic voltammetric[٢٥] . Derivative spectrophotometric technique and chemometric methods[٢٦-٢٧] The estimation of paracetamol and orphenadrine citrate in a multicomponent pharmaceutical dosage form by spectrophotometric method has been reported. Because of highly interference in the spectra and the presence of non-linearity caused by the analyte concentrations which deviate from Beer and Lambert's law, partial least-squares (PLS) and artificial neural networks (ANN) techniques were used for the calibration[٣] A micellar electrokinetic chromatography (MEKC) method was established for determination of paracetamol (PARA) and chlorpheniramine maleate (CPM) in cold tablets. Separation of both drugs rapid and efficient[٤]

cephalexin monohydrate

Cephalexin monohydrate contains not less than ٩٥,٠ per cent and not more than the equivalent of ١٠١,٠ per cent of (٦*R*,٧*R*)-٧-[(*R*)-٢-amino-٢- phenylacetamido]-٣-methyl-٨-oxo-٥-thia-١-azabicyclo[٤,٢,٠]oct-٢ - ene-٢-carboxylic acid, calculated with reference to the anhydrous substance. The empirical formula of cephalexin was ($C_{١٦}H_{١٧}N_٣O_٤$ S, $H_٢O$), and the molecular weight (٣٦٥,٤g/mol) [٢]. Cephalexin occurs as white, or almost white, crystalline monohydrate powder. It is soluble in water, practically insoluble in alcohol and in ether, resistant to acid and well absorbed orally. Several different methods have been used for determination of cephalexin monohydrate including; High-performance liquid chromatographic[٢٨-٣١]. A capillary zone electrophoresis method[٣٢]. Fluorimetric Method [٣٣،٣٤].And Electroanalysis such as Polarography and voltametry[٣٥،٣٦]. And there are several methods used for the determination of cephalexin[٣٧]. Recently Simultaneous Spectrophotometric determination using of Chemometrics methods[٣٨]

The H-point standard addition method[٥] (HPSAM) permits both proportional and constant errors produced by the matrix of the sample to be corrected directly. This method was based on the principle of dual wavelength spectrophotometry and the standard addition method. The greatest advantage of HPSAM is that, it can remove the errors resulting from the presence of an interfering and blank reagent. Although HPSAM could remove the error resulting from the sample matrix, it cannot remove the constant error resulting from other components in the system. The requirements for the application of the method is that if necessary to work only at two wavelengths where the analytical signal due to the one of the species is constant and for another one to be as different as possible. By plotting the analytical signal versus the added analyte concentration, two straight lines are obtained that have a common point with coordinates H ($-C_H$, A_H), where $-C_H$ is the unknown analyte concentration and A_H the analytical signal due to the interfering species.

Recently H.point standard addition (HPSAM) were used for simultaneous determination of binary mixture [٣٩-٤٧]. In this work HPSAM

Derivative spectroscopy[٤٨] were employed for the resolution of binary mixtures of paracetamol and cephalexin . The suggested methods were successfully applied to the determination of these analytes in synthetic mixtures.

Experimental

Instruments and Equipments:

Double-beam UV-Visible spectrophotometer model (UV-١٦٥٠ PC) SHIMADZ (Japan), interfaced with computer via a SHIMADZU UV probe data system program (Version ١,١٠), using ١,٠٠ cm quartz cells, Ultra sonic devise (ultrasonicator) for dissolving samples, (SONOREX), (W. Germany), Ultra pure water manufacturing devise, (TORAYPURE), model LV-٠٨ (Japan).

Chemicals

Standard drugs: cephalexin monohydrate ($C_{١٦}H_{١٧}N_{٣}O_{٤}S.\ H_{٢}O$; F.W. ٣٦٥,٤) and Paracetamol ($C_{٨}H_{٩}NO_{٢}$.;F.W ١٥١,٢) were provide from the State Company of Drug Industries and Medical Appliances (IRAQ-SDI-Samara). All drugs were used as working standards without further purification and analyzed to one of the official methods or reported methods to determine their purity and compliance with the requirements.

Preparation of Stock and working Standard Solutions

١-Stock solutions (٥٠٠ or ٢٥٠ mg/L) of standard were prepared by dissolving an accurately weighed amount (٥٠ or ٢٥ mg) of the studied drugs in about ٨٠ ml of the deionized water ١٠٠ ml volumetric flask .using ultra sonic devise (ultrasonicator) for dissolving samples, The solution is then made up to the volume with deionized water the stock solutions were completed quantitatively with the deionized water to

obtain the suitable working standard solutions according to the linear calibration range for each drug.

٢- Two series of pure single standards drugs prepared by dilution from stock solutions with the deionized water to prepare suitable constrictions (٢- ٧٠ mg/L)

٣- Solutions for binary mixtures of standard drugs cephalexin monohydrate solutions and paracetmol were prepared by two series;

First series of mixture solutions were prepared by using a fixed concentration of (٣٠ mg/L) for ceph with different concentrations (٢, ٥, ١٠, ٢٠, ٣٠, ٤٠, and ٥٠ mg/L) of para, second series of mixture contain a fixed concentration (٣٠ mg/L) of para with different concentration of (٢, ٥, ١٠, ٢٠, ٣٠, ٤٠, and ٥٠ mg/L) of ceph.

Results and Discussion

Derivative spectroscopic Methods:

Normal spectrum can not be used to determine each of para and ceph present in mixture, due to interference between the spectra, as shown in Fig.١a. Therefore, UV derivative can be used in this case**.** As shown in Fig.١ and ٢, first derivative also can not used to determine ceph in the presence para by using zero crossing method .[٢٢]

Fig.٣ shows Second Derivative (^{٢}D) for ٢-٥٠ mg/L para spectra and ٣٠ mg/L zero crossing ceph. at valley ٢٥٠. nm and ^{٢}D spectra for ٢-٧٠ mg/L ceph.with ٣٠ mg/L para zero crossing at ٢٩٠,٢ nm for ceph., which were they are suitable for measuring para and ceph respectively.. The calibration curve of ^{٢}D with the range of concentrations (٢-٦٠ mg/L para) at ٢٥٠,٥ nm gave a linear equation with slope and the correlation coefficient and the relative errors for the mixtures for each drug were listed in Table١ and ٢.. The relative standard deviation for each concentration represents an average of at least three measurements is between ٠,٧٨١- ١,٠٦٣. Cephalexin can be determined in the presence of para using ^{٢}D spectrum at ٢٩٠,٢ nm. The calibration curve of ^{٢}D for

standard ceph solutions was ranged from ٢ to ٧٠ mg/L at ٢٩٠,٢ nm gave a linear equation with slope and the correlation coefficient and the relative errors for the mixtures each drug were listed in Table١ and ٢. and the relative standard deviation for each concentration represents an average of at least three measurements. is between ٠,٧٥٧- ١,٢٠٧.

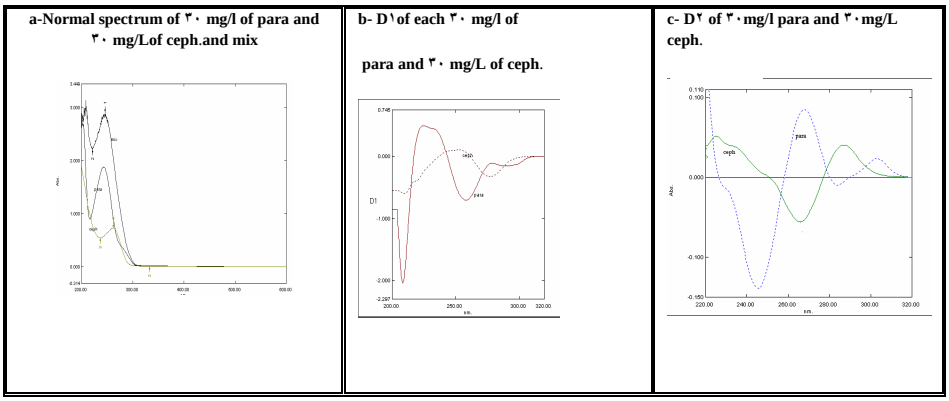


Fig.١: The spectra: a- normal spectra of para and ceph Δλ=٥, b- D١ of each para and ceph S=١٠, Δλ=٥, c- D٢ of each para and ceph S=٢٠, Δλ=٥.

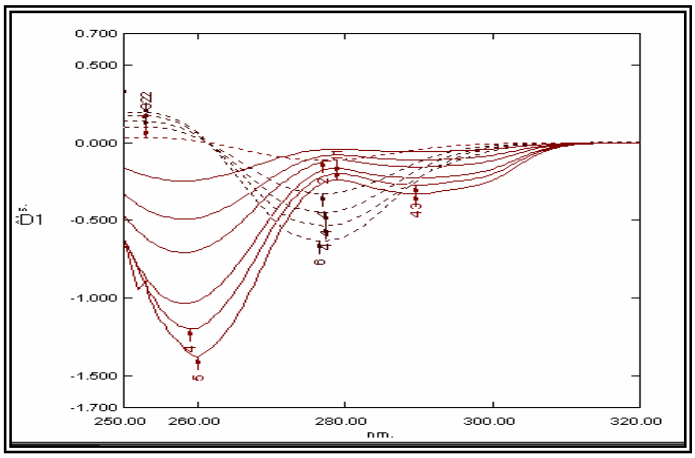


Fig. ٢- ١D spectrum for ١٠-٥٠ mg/L para and ١D spectra for ١٠-٦٠ mg/L ceph

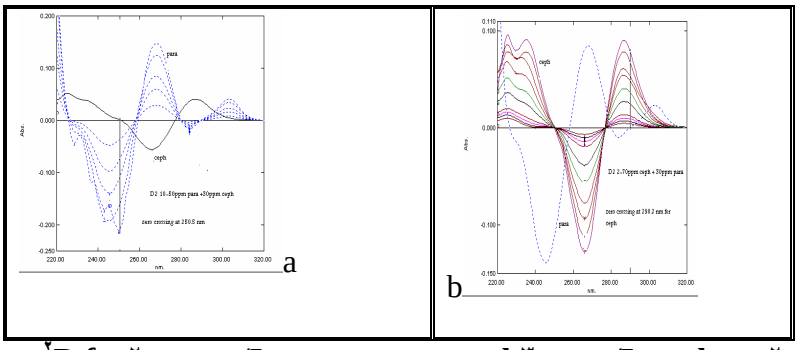


Fig.٣- a-^{٢}D for٢-٥٠ mg/L para spectra and ٣٠ mg/L ceph. at ٢٥٠,٥. b-^{٢}D spectra for ٢-٧٠ mg/L ceph.with ٣٠ mg/L para zero crossing at ٢٩٠,٢ nm for ceph

Table١: The Methods using for determination binary mixture para and ceph

Drug	Concentration range mg/L	Method		Equation	Relative error for ٥٠٪ mixture	r
para	٢-٦٠	^{٢}D	Valley=٢٥٠,٥	Y=٠,٠٠٤٣X + ٠٠٧٤	٠,٧٥٧	٠,٩٩ ٩٦
ceph	٢-٧٠	^{٢}D	Peak=٢٥٠,٥٠	Y=٠,٠٠١١X + ٠٠١٣	١,٢٠٧	٠,٩٩ ٩٥

Table-٢: Relative error of ceph in the presence of para and the relative error of para in the presence of ceph using ^{٢}D spectrum at valley ٢٩٠,٢ nm and ٢٥٠,٥ respectevely.

Ceph + amox mixtue	Ceph found* rmg/L	Relative error%	para + ceph mixture	Para found*	Relative error
١٠ ceph+٠٠ para	٩,٨٣١	-١,٦٩٠	١٠ para.+٠٠ ceph	١٠,٢١٢	+٢,١٢٠
٢٠ ceph+٠٠ para	١٩,٨٤٨	-٠,٧٦٠	٢٠ para.+٠٠ ceph	٢٠,٢١٨	+١,٠٩٠
٣٠ ceph+٠٠ amox	٣٠,٢٧٧	٠,٩٢٣	٣٠ para.+٠٠ ceph	٣٠,٢٦١	+٠,٨٧٠
٤٠ ceph+٠٠ para	٣٩,٦٤٥	-٠,٨٨٨	٤٠ para.+٠٠ ceph	٤٠,٣٢٨	+٠,٨٢٠
٥٠ ceph+٠٠	٤٩,٨٧٥	-٠,٢٥٠	٥٠ para.+٠٠ ceph	٥٠,٢٩٧	+٠,٥٩٤

amox					
١٠ceph +٣٠ para	٩,٦١٢	-٣,٨٨٠	١٠ para + ٣٠ ceph	١٠,١٢٣	+١,٢٣٠
٢٠ceph +٣٠ para	١٩,٨٩٦	-٠,٥٢٠	٢٠ para + ٣٠ ceph	٢٠,٣٢٤	+١,٦٢٠
٣٠ceph +٣٠ para	٣٠,٢٢٧	+٠,٧٥٧	٣٠ para + ٣٠ ceph	٣٠,٣٦٢	+١,٢٠٧
٤٠ceph +٣٠amox	٣٩,٩٢٧	-٠,١٨٣	٤٠ para + ٣٠ ceph	٣٩,٨٤٤	-٠,٣٩٠
٥٠ceph +٣٠ para	٤٩,٩٣٤	-٠,١٣٢	٥٠ para + ٣٠ ceph	٤٩,٩٢٠	-٠,١٦٠
٣٠ceph + ١٠ para	٣٠,١٢٩	+٠,٤٣	٣٠ para + ١٠ ceph	٣٠,٢٦٣	+٠,٨٧٧
٣٠ceph + ٢٠ para	٣٠,١١٥	٠,٣٨٣	٣٠ para + ٢٠ ceph	٣٠,٣٤٥	+١,١٥٠
٣٠ceph + ٤٠ para	٢٩,٨١٣	-٠,٦٢٣	٣٠ para + ٤٠ ceph	٣٠,٤٥٢	+١,٥٠١
			٣٠ para + ٥٠ ceph	٣٠,٧٦٣	+٢,٥٤٣

- **each value is the mean at least three measurements**

Applying HPSAM

In the proposed systems Para and Ceph are the analyte and interfering, respectively. As seen in Fig. ١, at the selected wavelengths of λ_1 = ٢٦٥ and λ_2 = ٢٥٧ nm, the Para signal increases linearly with the increase in concentration of para, whereas the ceph signal does not change with the increase in analyte concentration. The concentration of para is determined by HPSAM using two wavelengths, λ_1 = ٢٦٥ and λ_2 = ٢٥٧ nm, at which the interfering species, ceph, should have the same absorbance. Known amounts of para are then consecutively added to the mixture. After each addition the absorbance (A) is measured at the two wavelengths, and expressed by the following equations[٤١].

$$A_{٢٦٥} = M_{٢٦٥}C_{para} + b_o + b \qquad (١)$$

$$A_{٢٥٧} = M_{٢٥٧}C_{para} + A_o + A' \qquad (٢)$$

The two straight lines obtained intersect at the H-point (para, ceph) (Fig. ٥).To achieve the simultaneous determination of ceph and para in a sample, several synthetic mixtures with different concentration ratios of para and ceph were analyzed using HPSAM as shown in fig ٦ and table ٣. Results of the analysis of the different mixtures in the proposed system revealed a dynamic range and of ٢-٣٥ and ٢-٥٠ mg/L for para and ceph, respectively. The relative error are between -٠,٦٠٥-١,٦٤ and -١,٢٣٠-١,٤٣ , for para and ceph respectively. RSD% ٠,١٥١-٥,٧٧٥ %

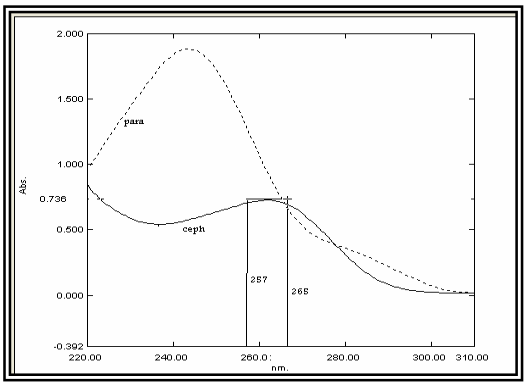


Fig.٤- normal spectra ٣٠mg/L ceph , ٣٠mg/L Para, at ٢٦٥ and ٢٥٧nm.

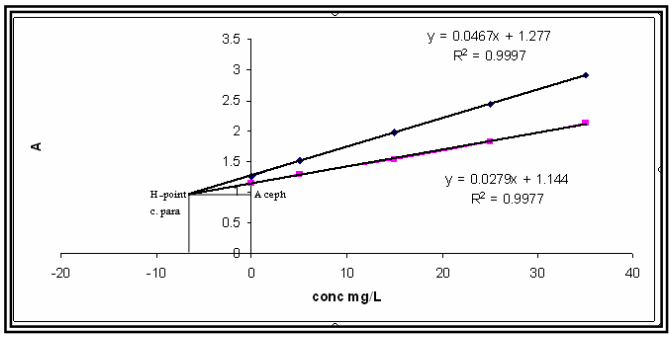


Fig ٥- H.PSAM for ٣٠ mg cephalexin with ٠,٠ – ٣٥ mg/L paracetmol at ٢٦٥ and ٢٥٧ nm.

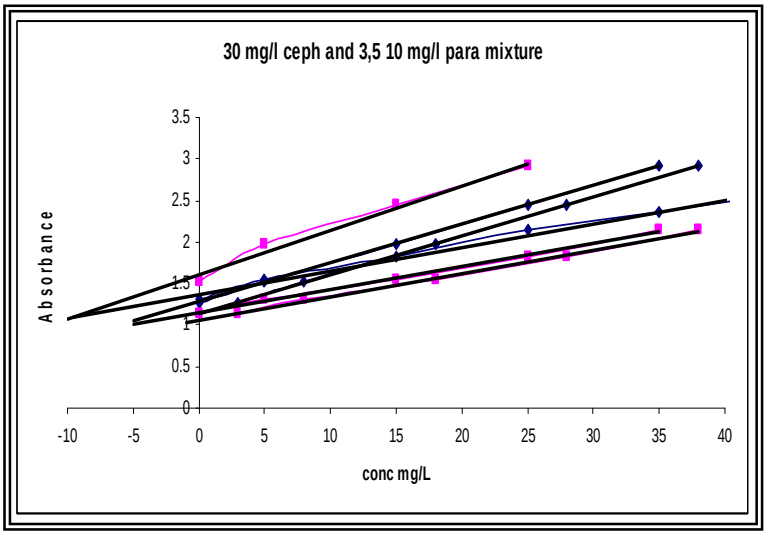


Fig. ٦ – H.point standard addition plot for simultaneous determination three mixtures of para and ceph with constant concentration of Ceph ٣٠ mg/L and the para concentration of ٢, ٥, ١٠ mg/L para respectively.

Table-٣: Relative error of ceph and para present in synthetic mixture using HPSAM..

Conc. Para +ceph	Para found* Mg/L	Relative error	Ceph found* mg/L	Relative error%
٢ para + ٣٠ ceph	٢,٠٢٤	+١,٢	٣٠,٢٥٤	+٠,٨٨٧
٥para + ٣٠ ceph	٥,٠٨٢	+١,٦٤	٣٠,٤٢٩	+١,٤٣٠
١٠ para + ٣٠ ceph	٩,٩٥٧	-٠,٤٣	٢٩,٨١٨	- ٠,٦٠٧
٢٠ para + ٣٠ ceph	١٩,٨٧٩	-٠,٦٠٥	٢٩,٦٣١	-١,٢٣٠

*** each value is the mean at least three measurements standard deviation ٠,٠٤٥٤-٠,١١٥٣**

Conclusion

The above results show that HPSAM and Derivative spectroscopy allow rapid, accurate and simple resolution of paracetamol and cephalexin mixtures. The HPSAM can be used in the multi components samples with matrix effects because standard addition method has capability of removing these effects.

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