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FOUR SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS DETERMINATION OF CARBAMAZEPINE AND LAMOTRIGINE IN BINARY MIXTURES AND URINE SAMPLES

(Penentuan Serentak Karbamazapin dan Lamotrigin Di Dalam Campuran Perduaan Dan Sampel Urin Melalui Empat Kaedah Spektrofotometrik)

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Abstract

In this work four different UV-spectrophotometric methods are described for simultaneous determination of antiepileptic drugs; carbamazepine (CBZ) and lamotrigine (LMT) in binary synthetic mixtures and urine samples without separation. First method was by solving the two simultaneous equations (SEQ) based on total absorbance according to Beer's law. Second was Dual wavelength (DWSP) method; Absorbance difference between 304 and 313 nm was measureable for CBZ but was zero for LMT. Likewise the absorbance difference between 282 and 290 nm was significant for LMT, and zero for CBZ. Third involved the use of zero- crossing first derivative method (ZCDSP) using the amplitudes at 308.9 and 286.6 nm for CBZ and LMT respectively. Ratio Derivative Spectrophotometry (RDSP) was the last. Here, the absorbance at different concentrations of CBZ or LMT, was divided, wavelength by wavelength, by the absorbance of a divisor, which was LMT standard for the analyte CBZ, and vice versa for LMT, (Divisor=2.0µg.mL^{-1}) in both cases. The amplitude of the derivative ratio spectra at 290 nm with wavelength interval ($\Delta\lambda$=6.0nm) and 328 nm ($\Delta\lambda$=4.0 nm) were selected for the determination of CBZ and LMT respectively. CBZ and LMT were simultaneously determined in synthetic mixtures and urine samples by the four methods giving good linearity, r^2 ranged between 0.9990 - 0.9997. Detection Limit (D.L) was mostly less than 0.4µg.mL^{-1},while in case of ZCDSP and RDSP were between 0.01-0.2 µg.mL^{-1} with wider linearity range (1-50 for CBZ and 1 - 80µg.mL^{-1} for LMT). A slightly lower sensitivity was observed when suppressing solution for urine analysis was used to remove interferences. The recoveries of CBZ and LMT in samples of urine of a healthy person spiked with the drugs and using urine of a healthy person as a blank were, in most cases, around (101.0% - 103.33%) and (98.33% - 102.16%) with RSD $\leq$ 3.61 and 3.63% for CBZ and LMT respectively. The recoveries using suppressing solution were (101.66% - 105.41%) and (94.56% - 101.0%) with RSD $\leq$ 2.43 and 3.62% for CBZ and LMT respectively. Statistical comparison of the results with the mixture of standard solutions using F, and t-tests showed no significant differences between each of the four methods at 95% C.L. The proposed methods were successfully applied for the determination of CBZ and LMT in binary mixtures and urine samples.

Keywords: dual wave length, zero crossing derivative, ratio derivative spectrophotometry, tegretol and lamotigine determination

Abstrak

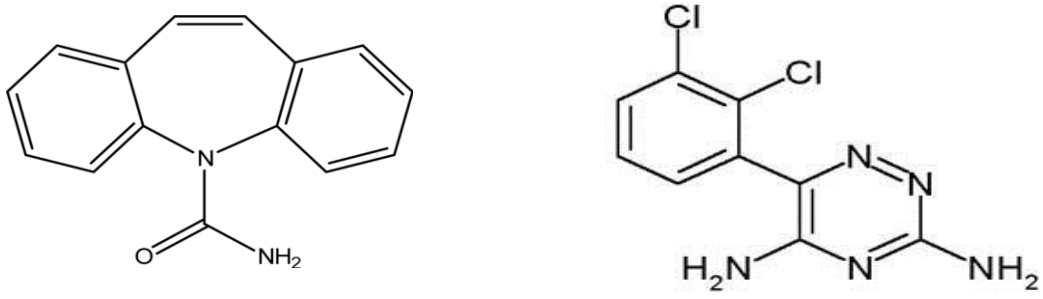
Dalam kajian ini empat kaedah spektrofotometri UV yang berbeza dijelaskan terhadap penentuan serentak dadah antiepilepsi; karbamazepin (CBZ) dan lamotrigin (LMT) dalam campuran sintetik perduaan dan sampel urin tanpa pemisahan. Kaedah pertama adalah dengan menyelesaikan dua persamaan serentak (SEQ) berdasarkan jumlah serapan menurut hukum Beer. Kedua adalah kaedah panjang gelombang dual (DWSP); Perbezaan serapan diukur antara 304 dan 313 nm terhadap CBZ manakala sifar bagi LMT. Begitu juga perbezaan serapan antara 282 dan 290 nm adalah penting untuk LMT dan set sifar untuk CBZ. Kaedah ketiga melibatkan penggunaan kaedah lintasan sifar terbitan pertama (ZCDSP) pada amplitud masing – masing 308.9 dan 286.6 nm untuk CBZ dan LMT. Kaedah spektrofotometri nisbah terbitan (RDSP) adalah yang terakhir. Serapan pada kepekatan yang berbeza CBZ atau LMT mengikut panjang gelombang, oleh serapan pembahagi, dimana larutan LMT adalah piawai untuk analit CBZ analit, dan sebaliknya. (Kepekatan Pembahagi = 2.0µg.mL^{-1}) dalam kedua-dua kes. Amplitud nisbah spektrum terbitan pada 290 nm dengan selang jarak gelombang ($\Delta\lambda$ = 6.0nm) dan 328 nm ($\Delta\lambda$ = 4.0 nm) masing – masing telah dipilih untuk

penentuan CBZ dan LMT. CBZ dan LMT telah ditentukan secara serentak di dalam campuran sintetik perduaan dan sampel urin oleh kesemua kaedah memberi kelinearan baik, r2 adalah antara 0.9990 - 0.9997. Had pengesanan adalah kurang daripada 0.4μg.mL^{-1}, manakala bagi kaedah lintasan sifar terbitan pertama dan spektrofotometri nisbah terbitan adalah di antara 0.01 – 0.2 μg.mL^{-1} dengan julat kelinearan lebih besar (1-50 bagi CBZ dan 1 - 80μg.mL^{-1} untuk LMT). Nilai sensitiviti diperhatikan lebih rendah apabila larutan tekanan digunakan terhadap analisis sampel urin untuk menghilangkan gangguan. Perolehan semula di dalam sampel urin bagi orang yang sihat yang dipaku bersama dadah CBZ dan LMT dan larutan pengosong dalam kebanyakan kes didapati pada julat (101.0% - 103.33%) dan (98.33% - 102.16%) dengan RSD 3.61 dan 3.63% masing – masing terhadap CBZ dan LMT. Perolehan semula menggunakan larutan tekanan adalah (101.66% - 105.41%) dan (94,56% - 101.0%) dengan RSD 2.43 dan 3.62% masing – masing terhadap CBZ dan LMT. Perbandingan statistik hasil kajian menggunakan ujian F dan ujian-t terhadap larutan piawai menunjukkan tidak ada perbezaan yang signifikan antara setiap satu daripada empat kaedah pada 95%. Kaedah yang dicadangkan telah berjaya diggunakan terhadap penentuan campuran perduaan CBZ dan LMT dan sampel urin.

Kata kunci: panjang gelombang dual, kaedah lintasan sifar terbitan pertama, kaedah spektrofotometri nisbah terbitan, penentuan tegretol dan lamotigine

Introduction

Carbamazepine (CBZ) or Tegretol and Lamotrigine (LMT) or Lamictal, Fig.(1) are two important antiepileptic drugs[1-4]. The CBZ is chemically known as 5H-dibenzo [b,f] azepine-5-carboxamide, is a highly lipophilic neutral tricyclic compound with a white to off white color almost odorless crystalline powder and has acid equilibrium constant (pKa of 13.9).It is slightly soluble in water but soluble in alcohol and acetonitrile[5,6]. In 1970, CBZ was approved in the United States for the management of seizure disorder in adults and in 1979 for children older than 6 years and remains widely used today[7,8]. LMT is chemically known as[6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine], has a broad-spectrum of the phenyltriazine class, chemically unrelated to other anticonvulsants[2-4]. It is a white to pale cream-colored powder and has a pKa of 5.7, slightly soluble in water but soluble in 0.1M H_2SO_4 [9], methanol and acetone[10].LMT is used as a monotherapy and as an adjunct with other antiepileptic drugs for treatment of partial and generalized tonic-clonic seizures. It is also used as tranquilizer and has also been studied in the treatment of neurological lesions[9].



(a) Tegretol (CBZ) (b) Lamotrigine (LMT)

Figure 1. The chemical structure of a. CBZ and b.LMT

Various analytical techniques have been reported in the literature to estimate CBZ and LMT in dosage forms and biological fluids including**:** Enzyme immunoassay methods [7, 8] for the estimation of CBZ and radioimmunoassay for the determination of LMT in human plasma were reported [11]. Most and a large number of methods for both CBZ and LMT analysis alone or together with other antiepileptic drugs or with their metabolites utilize, mainly HPLC techniques, and other chromatographic methods. Because of that,only those references appeared after 2010 are cited here[12-20]. Electroanalytical methods [1, 21-29] mostly voltammetric and few Ion- selective electrode methods, particularly for CBZ and rather few for LMT have been reported. Only few spectrophotometric methods,

both absorption and emission, were found reported for the determination of CBZ[30-35] and LMT[3,9,10,36-42] in pure forms or in pharmaceutical preparations. In a previous paper, Najib and Aziz [34] have developed two absorption methods for the determination of CBZ. In the present work four methods were tested for the determination of CBZ and LMT, in synthetic mixtures and human urine. For the best of the authors' knowledge, none of the published spectrophotometric methods have attempted the determination of these drugs together in a mixture and not by any of the developed methods in the present work. The methods studied in this work were; Calculation, dual wave length, zero crossing derivative and ratio derivative methods.

Materials and Methods

Apparatus and software

All spectra and absorbance measurements were recorded with (CECIL, CE9500, super aquarius) and (Perkin Elmer, Lambda 25 UV/VIS) double beam Scanning Spectrophotometers with 1.0cm matched quartz cells. Epson Printer LQ-2180, Grapher software (7.1.2005) Patch.msp, Microsoft office Excel 2007, Micropipettes from Gilson, France (variable and fixed).

Chemicals, Reagents and Drugs

All chemicals used were of analytical reagent grade, distilled water (D.W) was used throughout all the experiments. The organic solvents chloroform (Thomas Baker 99%), carbon tetrachloride (FISONS 95%), diethyl ether (Gainland Chemical Company 99.5%), acetone (Gainland Chemical Company 99.97%), n-hexane (Riedel de-Haen 97 %), cyclohexane (Merck 99.5%), methanol (Alpha chemika 99.9%) and ethanol (Scharlau 99.9%) without further purification. Pure and standard powder of lamotrigine was obtained from the (Hikma company, Jordan). Standard carbamazepine as a pure compound was obtained from (Samarra drug-company-Iraq). Tablets of the drugs from different origins were purchased from local market.

Stock standard solutions:100 μg/mL of each of CBZ and LMT were prepared by dissolving accurately weighed 0.01g of each drug in ethanol: water mixture (2:1) in 100 mL volumetric flasks. Different solutions of the compounds needed were prepared in the usual way.

Deproteinization of urine samples were performed [43] by taking 2.0 mL of spiked urine in a beaker. A portion of 1.2 mL from 0.15 M $Ba(OH)_2$ were followed by 1.2mL of 2.5% w/v $ZnSO_4.7H_2O$ and mixed well. Then filtered through a filter paper, no.41 (pore size, 20-25μm, particle retention) from Whatman. The filtrate was diluted to 50 mL by D.W in a volumetric flask. Unless otherwise stated, the blank was always ethanol: water mixture (2:1).

Results and Discussion

Choice of the organic solvent and the absorption spectra

The organic solvents chloroform, carbon tetrachloride, diethyl ether, acetone, n-hexane, cyclohexane methanol and ethanol were studied for their suitability, as shown in Table 1.

Volumes of 0.6 mL of CBZ and 1.0 mL of LMT, completed with each solvent to 10 mL were tested by recording their overlaid absorption spectra over the range 200-400 nm using corresponding solvent as a blank. The only suitable solvent among them, regarding both solubility and smooth output spectra, was ethanol. Among different ratios of this solvent with water, the mixture of ethanol: water (2:1) was chosen as the most suitable solvent, Fig. 2. This figure shows that (λ_{max}) were 286 and 308 nm for CBZ and LMT, respectively. The figure also shows partial spectral overlaps which did not allow their direct individual spectrophotometric analysis when present together in a mixture.

Table 1. Choosing best solvents for dissolving CBZ and LMT

Solvents	Carbamazepine	Lamotrigine	Results
Chloroform	Soluble	Soluble	Not suitable; both CBZ and LMT gave noisy absorption spectra
n-Hexane	Slightly Soluble	Insoluble	Not suitable
Diethyl ether	Soluble	Soluble	Not suitable; for CBZ due to noisy absorption spectrum.
Acetone	Soluble	Soluble	Not suitable for both CBZ and LMT. Noisy absorption spectra were obtained
CCl_4	Insoluble	Insoluble	Not suitable
Cyclohexane	Slightly Soluble	Slightly Soluble	Not suitable
Ethanol	Soluble	Soluble	Suitable
Methanol	Soluble	Soluble	Suitable

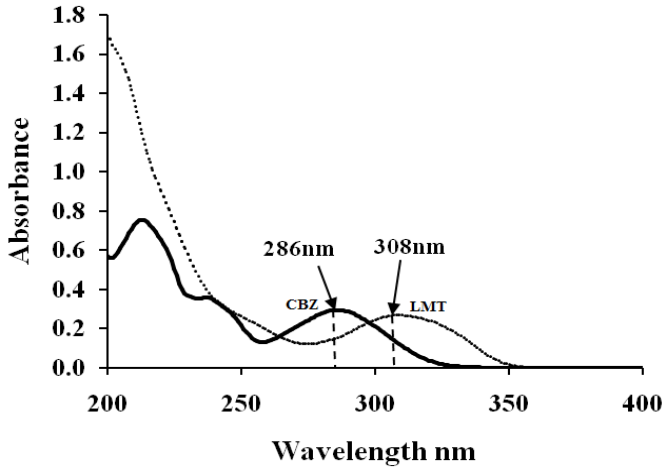


Figure 2. Zero-order absorption spectra of 6.0 µg/mL CBZ (——) and 10 µg/mL LMT (·····) using mixture of ethanol: water (2:1) as a solvent and blank.

The determination of CBZ and LMT in binary mixtures without separation

The calculation method (SEQ)

Although this method might be considered as a straight forward attempt, but it has not been tried before for these drugs and perhaps for others; it has been used for the determination of binary mixtures of textile dye[44]. The concentration C of each component of the sample will be calculated from the additive absorbance *A* in Beer's Law at λ_{max} of each of (a)and (b) following simultaneous equations, assuming CBZ = a and LMT = b.

$$A_{\lambda a\max} = \varepsilon_{\lambda a\max} C_a + \varepsilon'_{\lambda a\max} C_b \quad (1)$$

$$A_{\lambda b\max} = \varepsilon_{\lambda b\max} C_a + \varepsilon'_{\lambda b\max} C_b \quad (2)$$

The four molar absorptivity **ε** values were calculated from individual standards of (a) and (b) using Beer's law. The concentrations of (a) and (b) will then be calculated from equations3 and 4.

$$C_a = \frac{A_{\lambda a\max}\varepsilon'_{\lambda b\max} - A_{\lambda b\max}\varepsilon'_{\lambda a\max}}{\varepsilon'_{\lambda b\max}\varepsilon_{\lambda a\max} - \varepsilon'_{\lambda a\max}\varepsilon_{\lambda b\max}} \quad (3)$$

$$C_b = \frac{A_{\lambda b\max}\varepsilon_{\lambda a\max} - A_{\lambda a\max}\varepsilon_{\lambda b\max}}{\varepsilon'_{\lambda b\max}\varepsilon_{\lambda a\max} - \varepsilon'_{\lambda a\max}\varepsilon_{\lambda b\max}} \quad (4)$$

Dual wavelength method (DWSP)

This method is based on the principle that absorbance difference between two points of the wavelength spectra of the mixture is directly proportional to concentration of the component of interest, while the unwanted compound gives a ΔA=zero[45,46]. In an easy way, the selective determination of both drugs CBZ and LMT in a binary mixture will become possible.

Selection of suitable wavelengths

Selection of suitable wavelengths plays an important role in dual wavelength method; to do this different wavelengths from Fig.(3) were tried for CBZ and LMT as shown in table (2). Using the absorbance values at 304 and 313nm for CBZ (where LMT has ΔA= zero) and the absorbance values at 282 and 290nm for LMT (where CBZ has ΔA= zero) were chosen for determination of CBZ and LMT respectively. It can be deduced from the table that, the wavelengths mentioned above gave best linear range and average recovery percent in the binary mixtures and urine samples.

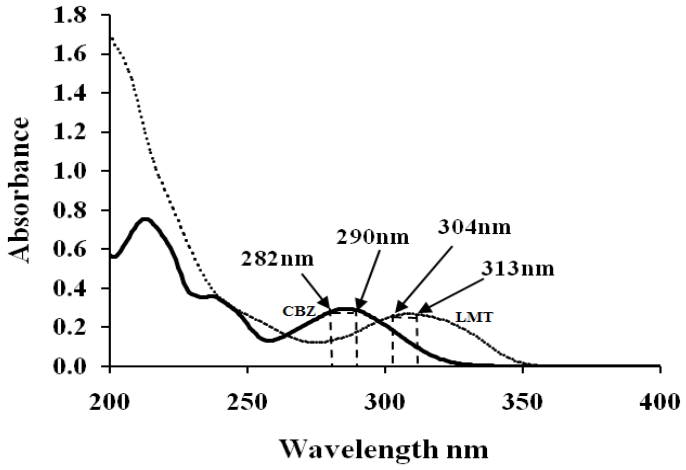


Figure 3. Zero-order absorption spectra of 6.0 µg/mL CBZ (——) and 10 µg/mL LMT (·····) using mixture of ethanol: water (2:1) as a solvent and a blank.

Zero-crossing first derivative spectrophotometric method (ZCDSP)

Zero-crossing derivative spectrophotometry involves measurement of the amplitude of the analyte at a wavelength at which absorbance of the unwanted species is nearly zero for different concentrations while the other component (the analyte) has appreciable absorbance (amplitude) [47,48].

Figure 4 shows first derivative UV- spectra of the same solutions of Figure (2). Derivatization of the zero-order spectra leads to an improvement of the spectral details and the resulting first derivative presents spectral features which can be used for simultaneous determination of CBZ and LMT. The figure shows that the signals of the first derivative of LMT are crossing zero at 309.8 nm and 275.6 nm and calibration curves for CBZ were linearly proportional to its concentration. The chosen wavelength, however, was found better to be at 309.8 nm. The same figure also shows the zero-crossing wavelength of CBZ at 286.6 nm, while at this wave length the derivative amplitude was found to be proportional to LMT concentration.

Table 2. Optimization of wavelength for simultaneous determination of CBZ and LMT by dual wavelength method

Compound	Wavelength nm	Linear range µg/mL	Slope	Intercept	Correlation coefficient
CBZ	255 and 297	3.0-15	0.0159	0.0014	0.9995
	300 and 319	6.0-17	0.0287	- 0.0049	0.9992
	250 and 306	3.0-16	0.0115	- 0.0029	0.9996
	304 and 313	3.0-21	0.0155	- 0.0017	0.9996
LMT	276 and 296	4.0-26	0.0101	- 0.0017	0.9993
	245 and 285	3.0-19	0.0155	- 0.0020	0.9994
	282 and 290	3.0-32	0.0041	-0.0005	0.9994

Selection of optimum instrumental conditions

The main instrumental parameters that affect the shape of the derivative spectra are the wavelength scanning speed and the smoothing of the resulting derivatives. Different scanning rates were tested and it was found that 25 nm/sec was a suitable scanning speed with band width of 2.0 nm. Several values of smoothing orders between (2-9 nm) were tested and the order 3.0 nm was selected due to its optimum signal, linearity range and recovery for synthetic mixtures of CBZ and LMT.

Effect of each of the two drugs on the peak height of the other

Two sets of standard solutions, the first was containing different concentrations of LMT (6.0-14 µg/mL) with constant concentration (3.0 µg/mL) of CBZ. The second contained between (3-5.0 µg/mL) of CBZ with (3.0 µg/mL) of LMT.

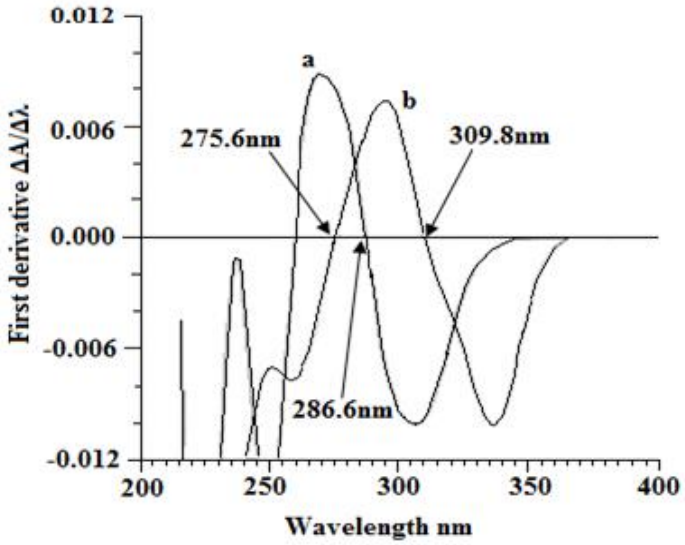


Figure 4. First derivative spectra of (a) 6.0 µg/mL CBZ and (b) 10 µg/mL LMT.

Their first derivative spectra, show that the heights at 309.8 and 286.6 nm were not affected by the presence of CBZ and LMT over the range of concentrations studied. According to previous optimizations, calibration curves for the determination of CBZ and LMT by measuring amplitude of the derivative spectra at 309.8 (Fig.5a) and 286.6 nm (Fig.5b) for both CBZ and LMT respectively, using (Grapher software (7.1.2005) Patch.msp),were found to be linear in the range of (1.0-50.0 and 1.0-80.0) µg/mL with (r^2=0.9995) and (r^2=0.9997) for both CBZ and LMT respectively. At lower concentrations, the first derivative amplitude could not be measured while at higher concentrations the first derivative amplitude was out of scale in both cases.

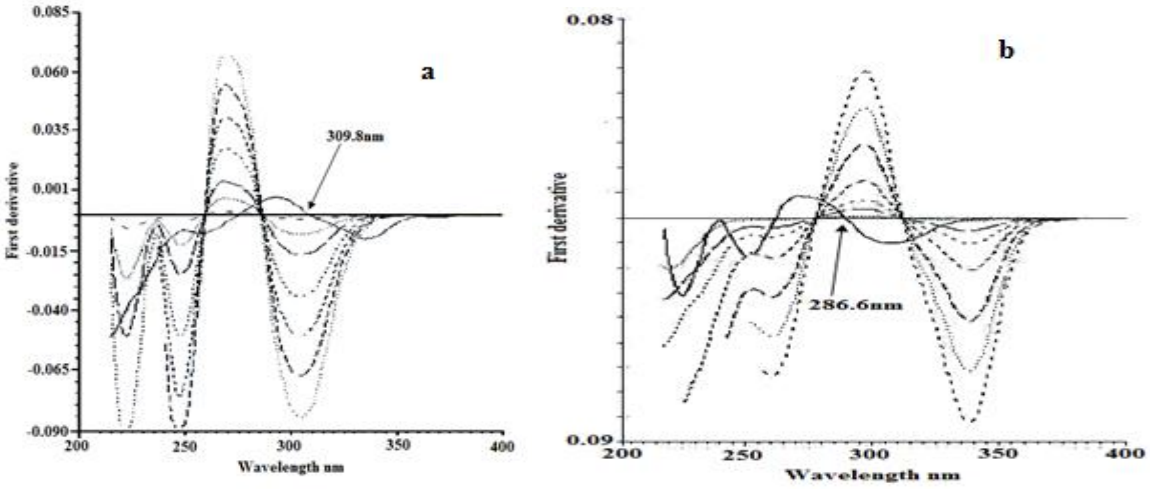


Figure 5. (a) First derivative absorption spectra of 10 µg/mL of LMT (———) and (1.0-50.0) µg/mL of CBZ (·····), (b) 6.0 µg/mL of CBZ (———) and (1.0-80.0) µg/mL of LMT (·····) using ethanol: water (2:1) as a blank (smoothing = 3.0 nm).

Ratio derivative spectrophotometric method (RDSP)

This is a modification of derivative spectrophotometric method based on the derivation of the ratio-spectra for resolving binary mixtures. The main advantage is the chance of performing easy measurements in correspondence of peaks; so that it permits choosing a wavelength at which the analytical signals of highest value (whether a maximum or minimum). Moreover, the presence of many maxima and minima will give the analyst more than one option to choose the most suitable wavelengths for the determination of one of the component in presence of the other which possibly interfere with the assay.

In this method, the absorption spectra of the mixture at each wavelength are divided by the absorption of a standard solution of one of the components, which is named a divisor. The first derivative of this ratio spectrum is obtained. The concentration of the other component is then determined from a calibration graph [49-50]. To apply this method for the determination of CBZ and LMT, it was necessary to optimize wavelength scanning speed, the concentration of the standard solution used as a divisor and the wavelength increment ($\Delta\lambda$) over which the derivative is obtained.

The effect of wavelength scanning speed was first studied. It was found that at high speed, noisy spectra were obtained while at low scanning speed, longer time was needed for measurements. Therefore medium scanning speed, 240 nm/min with spectral band width 2.0 nm were chosen for measurements. Different concentrations of CBZ and LMT standards were examined as divisors for each other. Several tests were carried out to study both the divisors concentrations and ($\Delta\lambda$). Increasing or decreasing the concentration of any one as the divisor, has made the resulting derivative values (hence, the slope of calibration graphs) proportionately decreased or increased, with potential variation of both sensitivity and linear range. Accordingly, best results for the divisors with minimal differences were found to be2.0-10 μg/mL in both cases. Outside these concentration ranges, the noise has greatly increased; with distortion and variation of the shape of curves and location of the peaks. For all subsequent measurements, a standard divisor 2.0 μg/mL for both CBZ and LMT were selected.

In Figure 6a and 7a, there are two series of ratio spectra of CBZ (from 1.0-50 μg/mL) and LMT (1.0-80 μg/mL) respectively. While Figs. (6b,7b), show the corresponding calculated first derivative ratio spectra of Figs, (6a,7a) using excel program. For the determination of CBZ by measuring amplitude at 266 and 290 nm, good linearity was observed but analytical signal and recovery percent were better at 290nm. For the determination of LMT, however, measuring amplitude at 328 nm, gave good linearity and recovery with low concentration of the divisor which was an important factor.

The $\Delta\lambda$ values of 6.0 nm for CBZ and 4.0 nm for LMT were selected as suitable wavelength intervals. Since in both cases the signals of the analytes (CBZ or LMT) that remain constant were not affected when the concentration of the variable analytes were increased up to 8.0 and 10 nm for CBZ and LMTrespectively. According to above optimizations, calibration curves were drawn for the determination of CBZ and LMT by measuring amplitude of the ratio derivative spectra at 290 and 328 nm against concentrations for both CBZ and LMT respectively. The calibration curves were linear in the range of 1.0-50.0 and 1.0-80.0 μg/mL, (r^2=0.9998) for both CBZ and LMT respectively.

Analysis of Synthetic Mixtures of CBZ and LMT by the Proposed Methods

Analyzing synthetic mixtures, other parameters not mentioned with the individual studies are given here. In order to demonstrate the validity and applicability of the proposed methods, recovery studies were performed. Results of table (3) which represent different composition ratios of CBZ and LMT, gave reasonable accuracies for the four methods, without any questionable data. Other data studied include, sensitivity, selectivity, accuracy and precision. Sensitivity represented by detection limit (D.L. μg/mL) according to equation,

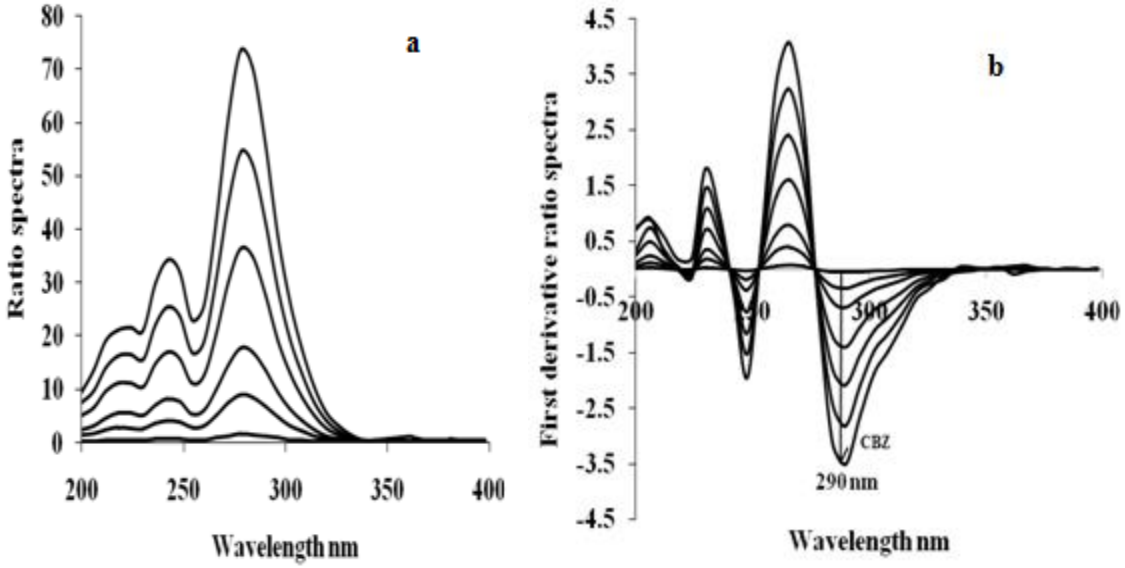


Figure 6. (a) Ratio spectra for 1.0, 5.0, 10, 20, 30, 40, 50 µg/mL solution of CBZ in ethanol: water (2:1) when 2.0 µg/mL LMT used as a divisor (Δλ= 6.0 nm).(b) is its first derivative.

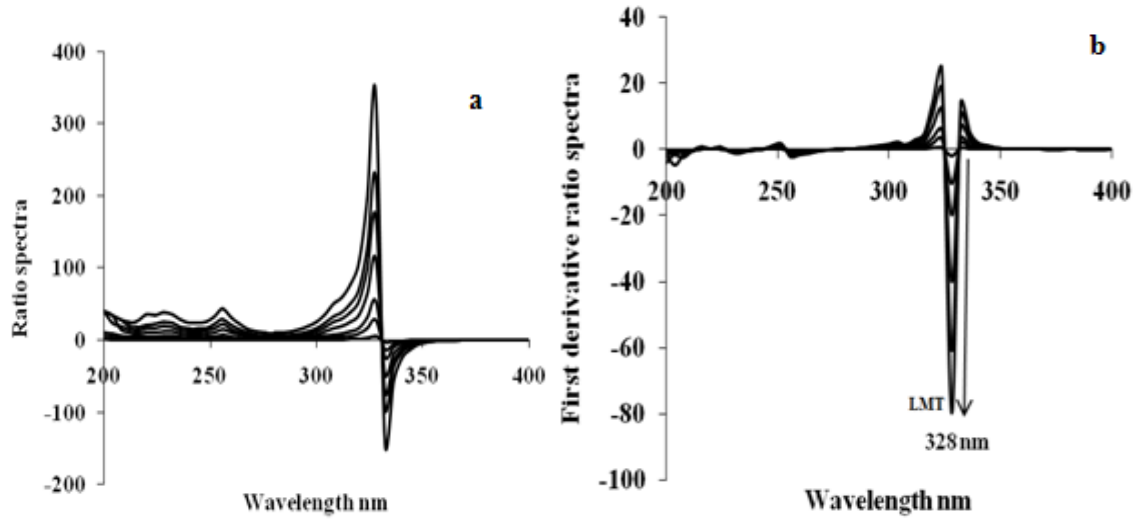


Figure 7. (a) Ratio spectra for 1.0, 5.0, 10, 20, 30, 40, 60, 80 µg/mL solution of LMT in ethanol: water (2:1) when 2.0 µg/mL CBZ used as a divisor (Δλ= 4.0 nm). (b) Its First derivative.

D.L =3.3σ/S, [σ = standard deviation of Blank signal and **S** = slope of the calibration curve] [51] for the methods, (SEQ, DWSP, ZCDSP and RDSP) were (0.12, 0.26), (0.11, 0.20), (0.09, 0.10) and (0.08, 0.03) for CBZ and LMT respectively. The precision of CBZ and LMT determination by the proposed methods was found by taking two samples of known concentration and their analytical signals were measured 10 times for the same sample, showing mainly, the precision of the instrumental measurements. The precision was also found on 10 times repeating the whole operation on the same sample; which confirms the precision of the whole procedure. The results, generally, indicate good precision, while the results of derivative methods show better precision than the other two methods.

The %Recovery and %Error for the determination of known concentrations of CBZ and LMT in mixtures made from their standard solutions were quite reasonable. The difference between the mean ($\overline{X}$) and the true value (μ) was tested for the existence of a systematic error in the results. The results were obtained by comparing the actual difference between ($\overline{X}$) and (μ); ($\overline{X}$ - μ) and the term (t.S/$\sqrt{N}$) at 95% confidence limit DOF = 2. From the results of t-test for the four methods, it can be concluded that the difference between ($\overline{X}$ - μ) and (t.S/$\sqrt{N}$) was not significant at 95% C.L, indicating the existence of too small or no significant systematic error.

Table 3. Analysis of synthetic mixtures of CBZ and LMT using the proposed methods.

Sample number	CBZ taken (μg/mL)	LMT taken (μg/mL)	SEQ %Recovery		DWSP %Recovery		ZCDSP %Recovery		RDSP %Recovery	
			CBZ	LMT	CBZ	LMT	CBZ	LMT	CBZ	LMT
1	8.0	3.0	96.50	95.30	95.00	95.20	96.89	96.50	97.04	97.15
2	7.0	3.0	100.20	96.62	97.60	100.30	97.02	101.20	97.42	99.37
3	6.0	3.0	97.09	96.80	96.70	98.10	98.06	100.80	96.63	97.83
4	5.0	3.0	96.00	96.70	96.60	95.90	97.65	98.80	98.66	101.65
5	4.0	3.0	98.70	97.50	98.80	99.10	102.80	103.20	97.51	101.60
6	3.0	3.0	99.00	98.30	98.70	101.40	99.93	97.02	98.50	99.11
7	3.0	4.0	97.40	98.50	97.50	99.60	98.52	99.72	100.60	98.90
8	3.0	5.0	97.34	98.65	103.30	97.80	99.30	97.04	101.77	100.15
9	3.0	6.0	98.96	101.80	101.00	97.30	99.79	98.89	100.90	101.20
10	3.0	7.0	98.45	103.30	102.30	98.40	99.40	100.40	103.70	102.30
11	3.0	8.0	99.71	104.00	104.20	98.60	101.30	100.20	99.34	99.54
12	3.0	9.0	99.06	103.70	103.70	99.00	100.30	100.80	101.90	100.30
13	3.0	10.0	102.30	97.60	103.00	97.50	103.60	101.13	101.20	101.90

Selectivity of the Methods

The interfering effects of most of the ions and compounds that expected to be present in urine and of other antiepileptic drugs such as phenobarbital (PHE) were examined and are shown in table (4 and 5). It can be seen from table (4) that phenobarbital, carbamazepine and lamotrigine least interfere with each other in case of ratio derivative but interfere more with dual wavelength method, and the effect was more severe in the presence of CBZ. In general the results are still quite acceptable. The interfering effects of the most possible ions present in urine on simultaneous determination of CBZ and LMT are shown in table (5). The study has aimed to find maximum quantity of the interference on a synthetic mixture of 3.0 μg/mL of CBZ and 6.0 μg/mL of LMT, which can cause

not more than ±5% error (tolerance level, T.L). The cations chosen were (Na^+, K^+, Ca^{2+} and Mg^{2+}) in the forms of (Cl^-, HPO_4^{2-} and NO_3^-). It was found that the effect was mostly due to creatinine, and uric acid which were present in urine at a high level in addition to some cations and anions. The effect of the cation interferences was confirmed by examination of the cations [Na^+, K^+ and Mg^{2+}] all in chloride form showing high T.L. The cations [Na^+, K^+] were not effective when they were in the form of HPO_4^{2-} while [Na^+, K^+, $Mg^{2+}$and Ca^{2+}] were more effective when they were in the form of (NO_3^-). This indicated that the effect of NO_3^- was much severe than chloride and phosphate ions. Table (5) also shows very small interferences of [urea, glucose, fructose, and sucrose] present in urine.

Removing of Interferences Using Suppressing Solution (S.S)

The basic principle of this idea is that those interferences which are expected to be present in urine sample are added to the sample and to the blank solutions. The net result is subtraction of their effect from the sample. This solution is added only when the urine sample is analyzed [34]. The suppressing solution was prepared by dissolving 0.2069 g (800 ppm K), 0.015 g Uric acid(150 ppm) and 0.025 g Creatinine (250 ppm) in 100 mL D.W. After several tests, it was found that a portion of 0.10 mL of this solution (S.S) was optimum for 3.0 µg/mL CBZ and LMT, to obtain correct analytical signal.

Simultaneous determination of CBZ and LMT in a synthetic and Urine Samples

The proposed methods were applied for simultaneous quantification of CBZ and LMT in synthetic and urine samples. To decrease the effect of interferences present in urine samples taken from healthy volunteers not taking any drugs, a 2.0 mL portion of fresh urine was spiked with a known amount of standard solutions of CBZ and LMT. After deproteinization, the dilution of the urine sample was necessary. Correct result was obtained when the filtrate of urine sample was diluted to 50 mL (25 folds dilution).

Table 4. The tolerance levels (T.L) of interferences from other antiepileptic drugs on synthetic mixtures of CBZ**:** LMT = (1.0**:**2.0) and effect of each of them on the individual concentration of 3.0 µg/mL CBZ and 6.0 µg/mL LMT, which causes not more than ±5% error.[T.L] = [Interference] / [Analyte]

Drugs name, as Analyte in the Mixture	**T.L (folds) SEQ**		**T.L (folds) DWSP**		**T.L (folds) ZCDSP**		**T.L (folds) RDSP**	
	CBZ	LMT	CBZ	LMT	CBZ	LMT	CBZ	LMT
CBZ	.....	+5	.....	+5	.....	+6	.....	+6
LMT	-3	.....	-2.5	.....	-3	.....	-3	.....
PHE	+10	-7	+12	-5	+14	-8	+16	-8

The analytes were determined by the present methods, with and without using (S.S) and %Recovery of the two methods was calculated from the calibration curves. The results obtained were statistically compared with those obtained with corresponding mixture of standard solutions of CBZ and LMT. Statistical analysis of the results, using variance ratio F-test and t-test, revealed no significant difference between the performance of the proposed methods and the result of mixtures of standard solution of CBZ and LMT since the calculated F-values and t-values at 95 % confidence level were less than the tabulated values.

Test for the presence of differences between the means of the four proposed methods with and without using (S.S) in this work ($urine_{(1)}$ and $urine_{(2)}$) has been performed. This was done by comparing the difference between the values ($\overline{X}_1 - \overline{X}_2$), with $t.S_{pooled} \times \sqrt{(N1+N2)/N1N2}$ at 95% DOF = 4. The results showed that:

$$(\overline{X}_1 - \overline{X}_2) < t.S_{pooled} \times \sqrt{(N1+N2)/N1N2}$$

meaning no significant difference between the performance of the proposed methods (with and without S.S).

Table 5. The tolerance levels of interferences on a synthetic mixture of 3.0 µg/mL of CBZ and 6.0 µg/mL of LMT, which causes not more than ± 5% error; [T.L] = [Int.] / [Analyte] folds

Cations	**Salts of cations**	**T.L (folds) LMT**	**T.L (folds) CBZ**
Na^+	NaCl	850	2200
K^+	KCl	860	2400
Mg^{2+}	$MgCl_2.6H_2O$	180	990
Na^+	Na_2HPO_4	>1300	>2600
K^+	K_2HPO_4	>1300	>2600
K^+	KNO_3	6.0	8.0
Ca^{2+}	$Ca(NO_3)_2.4H_2O$	35	19
Mg^{2+}	$Mg(NO_3)_2.6H_2O$	27	18
Creatinine	$C_4H_7N_3O$	21	15
Glucose	$C_6H_{12}O_6$	280	1300
Urea	$(NH_2)_2CO$	410	800
Fructose	$C_6H_{12}O_6$	230	780
Sucrose	$C_{12}H_{22}O_{11}$	320	950
Uric acid	$C_5H_4N_4O_3$	3.0	2.0

Precision and Accuracy of the Methods:

To evaluate the precision and accuracy of the proposed methods, a known amount of the standard CBZ and LMT concentrations were spiked into the urine samples simultaneously determined by the four proposed methods. The accuracy as (%Recovery), is presented in table (6) showing generally good to reasonable results. The precision as percent relative standard deviation (%RSD), was determined by analyzing two series of ten urine samples having spiked with mixtures of CBZ and LMT (5.0:3.0) µg/mL and (3.0:6.0) µg/mL each. The low values of the %RSD, ranged between (0.63 -3.63) indicated high precision.

The accuracy of the derivative methods was better than the other two methods which may be due to the effect feature of the derivative methods. Non spiked urine was used as a blank while in the second method the effects of interferences were minimized by using the suppressing solution. The %Error in case of CBZ and LMT (5.0:3.0) µg/mL each, of dual wavelength method was slightly more than 5% only in one case for CBZ and another for LMT. The reason of this error may be due to the quantity of 0.1mL S.S which was thought not to be sufficient to minimize the effect of interferences. But the error has not increased to such an extent needing further treatment, and has not been repeated again during the whole process. The difference between the mean ($\overline{X}$) and the true value (µ) was performed to test for existence of a systematic error in the results as shown in table (6). The results were obtained by comparing the actual difference between ($\overline{X}$) and (µ); ($\overline{X}$ - µ) and the term (t.S/$\sqrt{N}$) at 95% confidence level DOF = 9.

The difference between ($\overline{X}$ - µ) and (t.S/$\sqrt{N}$) was significant at 95% C.L in both methods, indicating the existence of a small systematic error. This was mainly due to the coexistences of the drugs and the effect of (S.S), which may affect one method more than another or has different effects on CBZ compared to LMT; but in general the error values were still satisfactory.

The test for the presence of differences between the means of any two pairs of the proposed methods in the mixture CBZ and LMT ratio (5.0:3.0) and (3.0:6.0) were determined by comparing the difference between the values ($\overline{X}_1$- $\overline{X}_2$), with the term $t.S_{pooled} \times \sqrt{(N1+N2)/N1N2}$ at 95% C.L DOF = 18.

The results showed no significant difference between the performance of the two proposed methods (with and without using S.S) for simultaneous determination of CBZ and LMT in urine samples.

Table 6. Application of the present methods on a synthetic and urine samples for CBZ and LMT mixtures, with and without using the Suppressing Solution (S.S).

Sample Type	**CBZ:LMT Mixing Ratio**	**% Recovery**							
		SEQ		**DWSP**		**ZCDSP**		**RDSP**	
		CBZ	**LMT**	**CBZ**	**LMT**	**CBZ**	**LMT**	**CBZ**	**LMT**
Synthetic	5:3	98.00	96.33	96.10	101.60	98.40	98.13	98.66	101.20
	3:3	101.00	101.33	98.66	103.30	101.20	101.60	98.50	99.11
	3:6	97.00	97.33	98.66	100.30	101.90	97.85	100.90	99.80
Urine 1 No S.S	5:3	98.80	98.00	104.10	101.60	101.00	98.83	104.40	97.00
	3:3	102.60	105.00	100.40	98.33	101.00	101.50	97.33	101.00
	3:6	104.00	102.00	102,60	103.60	102.80	102.70	102.70	98.16
Urine 1 With S.S	5:3	103.80	96.00	106.00	94.74	103.00	97.00	103.40	96.70
	3:3	100.60	95.33	102.20	103.50	100.30	97.66	102.30	98.57
	3:6	101.60	101.60	102.20	95.66	104.70	99.66	100.50	101.40
Urine 2 No S.S	5:3	102.00	95.33	100.20	101.60	103.80	100.30	96.60	100.30
	3:3	104.60	104.36	102.60	103.30	102.00	102.60	96.00	103.70
	3:6	101.30	104.30	100.30	99.66	100.60	101.00	102.00	97.50
Urine 2 With S.S	5:3	101.00	95.00	97.76	96.49	100.80	96.33	99.60	95.66
	3:3	103.00	95.60	102.20	100.90	103.60	101.30	101.30	96.66
	3:6	101.30	98.83	104.10	96.50	104.90	98.16	103.00	100.30

Conclusion

Four sensitive and simple UV-Spectrophotometric methods were developed for simultaneous determination of antiepileptic drugs, carbamazepine (CBZ) and lamotrigine (LMT), in binary mixtures and urine without separation from each other. Interferences expected to be present in the urine samples were removed by addition of a suppressing solution to both samples and blank solutions or by using urine of a normal person not taking the drugs as a blank. The proposed methods were also selective for simultaneous determination of the two drugs in the presence of other co-administered antiepileptic drugs, such as phenobarbital. The suppressing solution was prepared from the salts of most interfering ions.

For validation, the determinations of CBZ and LMT in mixtures by the proposed methods were performed using their standards. Statistical analysis showed no significant difference between them at 95% C.L. Visible spectrophotometric methods published in the literature are only applicable for the determination of CBZ and LMT in tablets. In the UV region literature methods are only applicable for simultaneous detection of CBZ and LMT in biological fluid after extraction or separation with aid of other complicated techniques like HPLC. While this work has succeeded to determine the mixture of the two drugs in urine samples without separation.

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