Spectrophotometric and standard addition methods for quantitative determination of dopamine hydrochloride and levodopa in tablets and ampoules

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Métodos espectrofotométricos y de adiciones estándar para la determinación cuantitativa de clorhidrato de dopamina y levodopa en comprimidos y ampollas

Mètodes espectrofotomètrics i d'addicions estàndard per a la determinació quantitativa de clorhidrat de dopamina i levodopa en comprimits i ampul·les

Recibido: 29 de mayo de 2008; revisado: 4 de marzo de 2009; aceptado: 10 de marzo de 2009

RESUMEN

Se describe un método espectrofotométrico sencillo, preciso y sensible para la determinación de clorhidrato de dopamina (DO·HCl) y levodopa (LD) tanto en formas puras como en sus formulaciones farmacéuticas comercialmente disponibles. La idea principal del método se basa en hacer reaccionar una disolución de DO·HCl o LD a pH 12, utilizando un tampón universal, con 4-aminoantipirina (4-AAP) para formar un producto de acoplamiento de color rosa con lmax = 475 (e = 1,46·104 l·mol-1·cm-1) o 454 nm (1,05·104 l·mol-1·cm-1) para los compuestos DO·HCl y LD, respectivamente. Antes de aplicar la ley de Beer, se optimizan diferentes condiciones experimentales, tales como el tiempo, la temperatura, la secuencia de adición y el pH. El método de la razón molar revela que se obtiene un producto de acoplamiento 1:1 [ingrediente activo]:[4-AAP]. Se examina si los excipientes habitualmente empleados como aditivos en los preparados farmacéuticos interfieren en el procedimiento propuesto. Se realizan las curvas de calibración usando DO·HCl y LD estándares, con porcentajes de recuperación de 98,62 – 102,9% y 97,40 – 101,5%, respectivamente. La ley de Beer es obedecida en el margen de concentraciones 37,90 – 170,6 y 49,30 – 221,8 mg/L para DO·HCl y LD, respectivamente. Se evalúa la reproducibilidad y la precisión del método, obteniendo SD = 0,04-0,22 y 0,05-0,15, y RSD = 0,06-0,16 y 0,02-0,10% para DO·HCl y LD, respectivamente. Estos resultados se comparan favorablemente con los de los métodos oficiales y publicados, tal y como apuntan los valores del test-t y del test F, indicando la posibilidad de aplicar este método espectrofotométrico en medidas de rutina.

Palabras clave: Espectrofotometría, método de las adiciones estándar, clorhidrato de dopamina, levodopa, tampón universal, 4-aminoantipirina, comprimidos, ampollas.

SUMMARY

A Simple, accurate and sensitive spectrophotometric method for determining dopamine hydrochloride (DO·HCl) and levodopa (LD) in either pure forms or in their commercially available pharmaceutical formularions is reported. The main idea of the method is based on reacting a solution of DO.HCl or LD at pH 12; using universal buffer, with 4-aminoantipyrine (4-AAP) to form pink coloured coupling dye product with λmax = 475 ($\varepsilon = 1.46 \times 10^4$ l·mol^{-1}·cm^{-1}) or 454 nm (1.05×10^4 l·mol^{-1}·cm^{-1}) for DO·HCl and LD drugs, respectively. Before carrying out Beer's law, different experimental conditions like time, temperature, sequence of addition and pH are optimized. The molar ratio method revealed a 1:1 [active ingredient]:[4-AAP] coupling product. The common excipients used as additives in pharmaceuticals are examined in our proposed procedure as interfering materials. The calibration curves were developed by using standard DO·HCl and LD with percent recovery of 98.62 – 102.9 and 97.40 – 101.5%, respectively. Beer's law was obeyed in the concentration range from 37.90 – 170.6 and 49.30 – 221.8 mg/L of DO·HCl and LD, respectively. The reproducibility and accuracy of the method was checked by the values of SD = 0.04-0.22 and 0.05-0.15 and RSD = 0.06-0.16 and 0.02-0.10% for DO·HCl and LD, respectively. The results compare favourably with those of official and reported methods as indicated by the t- and F-test values, indicating the possibility of applying this spectrophotometric method in routine measurements.

Key words: Spectrophotometry, standard addition method, dopamine hydrochloride, levodopa, universal buffer, 4-aminoantipyrine, tablets, ampoules.

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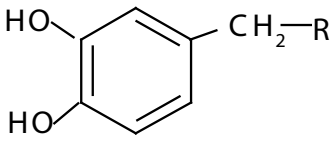
RESUM

Es descriu un mètode espectrofotomètric senzill, precís i sensible per a la determinació de clorhidrat de dopamina (DO·HCl) i levodopa (LD) tant en formes pures com en les seves formulacions farmacèutiques comercialment disponibles. La idea principal del mètode es basa en fer reaccionar una dissolució de DO·HCl o LD a pH 12, utilitzant un tampó universal, amb 4-aminoantipirina (4-AAP) per formar un producte d'acoblament de color rosa amb lmax = 475 (e = 1,46·104 l·mol-1·cm-1) o 454 nm (1,05·104 l·mol-1·cm-1) per als compostos DO·HCl i LD, respectivament. Abans d'aplicar la llei de Beer, s'optimitzen diferents condicions experimentals, tals com el temps, la temperatura, la seqüència d'addició i el pH. El mètode de la raó molar revela que s'obté un producte d'acoblament 1:1 [ingredient actiu]:[4-AAP]. S'examina si els excipients habitualment emprats com additius en els preparats farmacèutics interfereixen en el procediment proposat. Es realitzen les corbes de calibratge emprant DO·HCl i LD estàndards, amb percentatges de recuperació de 98,62 – 102,9% i 97,40 – 101,5%, respectivament. La llei de Beer és obeïda en el marge de concentracions 37,90 – 170,6 i 49,30 – 221,8 mg/L per a DO·HCl i LD, respectivament. S'avalua la reproduïbilitat i la precisió del mètode, obtenint SD = 0,04-0,22 i 0,05-0,15, i RSD = 0,06-0,16 i 0,02-0,10% per a DO·HCl i LD, respectivament. Aquests resultats es comparen favorablement amb els dels mètodes oficials i publicats, tal i com apunten els valors del test-t i del test F, indicant la possibilitat d'aplicar aquest mètode espectrofotomètric en mesures de rutina.

Mots clau: Espectrofotometria, mètode de les addicions estàndard, clorhidrat de dopamina, levodopa, tampó universal, 4-aminoantipirina, comprimits, ampul·les.

I. INTRODUCTION

Dopamine and dopamine derivatives were a group of biogenic amines possessing a 3,4-dihydroxy substituted phenyl ring (Figure 1).



DO; R: CH_2 CH_2 (NH_2)
LD; R: $CH(NH_2)COOH$

Fig. 1. *Structural formulae of the investigated dopamines.*

They considered as a type of hormones widely spread in animals and had also been detected in 44 plant families [1,2]. They were participated in regulation of a wide variety of physiological functions and released in small amounts. Over the years, catecholamines and their metabolites remained an important laboratory aid in the diagnosis of many physiological and psychiatric diseases. They also seemed to be a central pharmacophore and well probably existed in future drugs, especially in those developed for psychiatry disorders and neurological activity [3]. They might act not only as transmitters from the neuro (endocrine) to the immune system but also as autocrine\paracrine mediators in immunocompetent cells and as transmitters between these cells and the nerves [4,5]. It was not until the late 1950's that dopamine was recognized as a mamalian neurotransmitter in its own right but the demonstration of its non-uniform distribution in the brain suggested that it might has a specific functional role for dopamine [6]. It had therapeutic uses as a cardiostimulant for the treatment of acute circulatory insufficiency and hypotention and important role in the pathogenesis or drug treatment of certain brain diseases e.g. Parkinson's disease and Schizophrenia [7].

Administration of LD was a characteristic feature for Parkinson's disease. It must be combined with certain amount from CD, which facilitate LD transport to the brain and converted to dopamine in the basal ganglia [8]. Several methods were applied on pharmaceutical preparations containing DO.HCl or LD depending on oxidation reaction [9,10]. Determination of certain catechol derivatives likes pyrocatechol, DO.HCl and LD in either pure form or in its pharmaceutical formulation was suggested spectrophotometrically [11] by interaction of diazotized p-nitroaniline with catechol derivatives in the presence of molybdate ions in acidic medium, and indirect kinetic specrtophotometrically [12] with periodate in acidic medium. On the other hand, the interaction of DO.HCl with sodium salt of chloramine-T and traces of copper (II) ions in a phosphate buffer medium of pH = 7 [13] or trioxalatoferrate (III) complex [14] allowed formation of coloured products that can be detected spectrophotometrically in pharmaceutical ampoules.

Chromatographic methods [15-17] were utilized or the determination of catecholamines in pharmaceutical and biological fluids. Flow injection analysis (FIA) system using tubular electrode was used in the determination of DO in pharmaceutical preparations [18,19]. Also, determination of levodopa and dopamine in pharmaceutical preparations using spectrophotometric methods were carried out [20-23].

The theory of active ingredient reaction with reagents illustrated as follows: different colorimetric methods described for phenol determination [24, 25] were based on the reaction between phenols and 4-AAP to form antipyrine dyes where 4-AAP was found to be the most sensitive, fastest and precise colourimetric reagents. 4-AAP reacted with phenolic

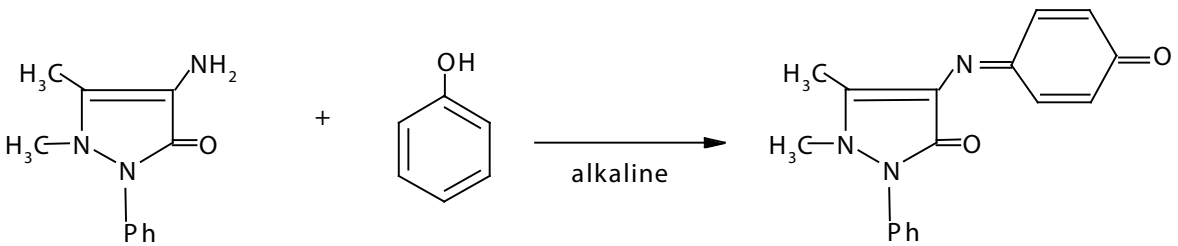


Fig 2. *Coupling reaction between phenol and 4-AAP.*

type compounds according to the reaction show in Fig. 2. The reaction product might be any colour from red to purple depends on the phenolic type compounds involved.

In continuation to our interest in microdetermination of these drugs under study [26], the aim of the present work is to describe the development of simple, sensitive and rapid spectrophotometric method for the determination of DO.HCl and LD depending upon the formation of their coupled products with 4-AAP at pH = 12. Different experimental conditions are carefully studied before applying Beer's law. The method was applied for determining DO.HCl and LD in pure form and pharmaceutical forms (tablets and ampoules) and the results obtained are compared well with those obtained by the official method.

II. EXPERIMENTAL

Apparatus

Absorption spectra measurements were recorded using Melton Roy Spectronic 601 and automated Perkin-Elmer Model Lambda 20 UV-Vis spectrophotometer in the wavelength range from 200 to 900 nm. The pH measurements were done using a Titrino Metrohm 716 DMS, connected to Metrohm stirrer. This instrument has a combined glass and reference electrodes used for pH measurements. For selecting accurate volumes, the calibrated micro-pipette (BRAND) of disposable plastic tips was used in the range 100 - 1000 µL. The water used was always de-ionized obtained from the instrument model Altra Clear SG-Wasser aufbereitung und Regenerierststion GmbH. HPLC was carried out using Perkin-Elmer instrument with binary LC pumb (250), C18 analytical column (250x4.60 mm) and uv-vis spectrophotometric detectoer (LC 290).

Materials

All reagents were of the analytical grade. Dopamine hydrochloride (DO.HCl) supplied from *Sigma,* while levodopa (LD), carbidopa (CD) and 4-aminoantipyrine (4-AAP) were purchased from *Fluka Chemie AG*. Sodium hydroxide was supplied from BDH. Glacial acetic, boric and phosphoric acids (90%) were purchased from Aldrich. Dopamine hydrochloride present in pharmaceutical preparations as dopamine Fresenius and Dopamine Pierre Fabre concentrate ampoules for infusion (200 mg/5 mL), where they were supplied from *Fresenius Kabi, Deutschland GmbH, D61346 Bad Homburg v.d.H*. and *Pierre Fabre Medicament Production, Boulonge, respectively*. Levocar tablets labeled 250 mg levodopa and 25 mg carbidopa were supplied from *Alpha Chem. Advanced Pharmaceutical Industries Co*. (ACAPI).

Reagents

10^{-2} M stock solutions of DO.HCl and LD were prepared in de-ionized water. The stock and dilute solutions were stored in dark bottles under cold condition (4 °C) in refrigerator. 10^{-2} – 10^{-3} M solutions of 4-AAP were prepared in 100 mL de-ionized water. All the required dilutions from reagents were prepared from stock one. A mixture of 0.04 M phosphoric, boric and acetic acids was titrated with 0.2 N NaOH to adjust the desired pH into 12 using pH-meter. 0.1 M Solution of interfering materials (glucose, ascorbic acid, catechol, phenol, pyrogallol, resorcinol, and hydroquinone) were prepared by dissolving the accurate calculated weight in 25 mL de-ionized water. 10^{-2} – 10^{-3} M solutions of the interfering materials were prepared by accurate dilution from the stock one.

LD-CD synthetic mixture was prepared to have 10:1 [LD]/[CD] ratio as that in Levocare drug where 0.1999 g and 0.0182 g of LD and CD, respectively, were dissolved in 100 mL de-ionized water. The concentration of each drug in the synthetic mixture becomes [LD] = 1.014×10^{-2} M and [CD] = 8×10^{-4} M.

Procedure

An aliquot containing 37.90-170.7 or 49.30-221.8 ppm of DO.HCl or LD, respectively, was transferred to 10 mL measuring flask, followed by adding 10^{-2} M 4-AAP. The pH was adjusted using universal buffer of pH = 12. The total volume was completed up to 10 mL. The mixtures were shacked well and allowed to stand at 30 ± 3 °C for 30 and 35 minutes, the pH was rechecked and the absorbance was measured at 475 and 454 nm for DO.HCl and LD, respectively, against de-ionized water as a blank. The calibration curves were obtained applying the same procedure using standard solutions of active ingredient.

Certain volume from dopamine ampoule (200 mg\5 mL) was diluted in 250 mL volumetric measuring flask to prepare 0.1 M solution. Then incubated in cold, dark bottles and away from oxygen air to prevent oxidation or decomposition

Twenty tablets of Levocare were accurately weighed and the average weight of one tablet was calculated. The tablets were crushed well to a fine powder. A portion of the powder equivalents to 250 mg LD-25 mg CD was dissolved in 100 mL de-ionized water. The resulting solutions were shacked well, filtered through a Whatmann No. 1 filter paper and washed with de-ionized water. The filtrate and washings were collected in 100 mL measuring flask then diluted to the volume with the same solvent. Aliquots of solutions of analyte were transferred to 10 mL measuring flask and quantitated applying our proposed procedure.

0.15 or 0.1 mL of DO.HCl ampoule or LD-CD tablet solution was mixed with serial known concentrations of active ingredients DO.HCl (1.1×10^{-4}- 6.5×10^{-4} mmole) or LD (3.75×10^{-4}- 8.75×10^{-4} mmole) solutions, respectively. The mixtures were mixed with 0.01 M 4-AAP and the procedure was completed as mentioned before.

RESULTS AND DISCUSSION

Determination of DO.HCl and LD using 4-AAP at pH =12

Optimum conditions affecting the reaction of DO.HCl and LD with 4-AAP are studied carefully.

By adding 4-AAP to DO.HCl and LD solutions in de-ionized water, colourless solutions were observed with λ_{max} = 278 nm ($\epsilon = 5.77 \times 10^4$ and 5.52×10^4 L $mol^{-1}cm^{-1}$) for DO.HCl and LD, respectively. The addition of universal buffer of different pH values (Table 1); from 2 to 12, to 4-AAP with DO.HCl or LD is studied and complete absorption spectra of these solutions from 200 to 900 nm are recorded. Colourless solutions were observed from pH 2 to 11 for both active ingredients with 4-AAP with λ_{max} = 260 ($\epsilon = 5.02 \times 10^4$ - 5.25×10^4 and 4.66×10^4- 4.89×10^4 L $mol^{-1}cm^{-1}$ for DO.HCl and LD, respectively), 279 ($\epsilon = 5.6 \times 10^4$ – 6.03×10^4 and 5.23×10^4- 5.6×10^4 L $mol^{-1}cm^{-1}$ for DO.HCl and LD, respectively) and 282 nm ($\epsilon = 6.28 \times 10^4$ – 7.1×10^4 and 5.88×10^4 - 5.92×10^4 L $mol^{-1}cm^{-1}$ for DO.HCl and LD, respectively). At pH = 12, a new pink coloured product is formed between DO.HCl or LD with 4-AAP with λ_{max} = 475 ($\epsilon = 1.46 \times 10^4$ L $mol^{-1}cm^{-1}$) or 454 nm ($\epsilon = 1.05 \times 10^4$ L $mol^{-1}cm^{-1}$), respectively (Table 1).

Applying the molar ratio method, it is found that DO.HCl or LD interacts with 4-AAP to form 1:1 [4-AAP]: [active ingredient] product under alkaline condition of pH = 12.

The effect of time on the reaction of DO.HCl or LD with 4-AAP is studied in the presence of universal buffer of pH = 12 and at λ_{max} = 475 or 454 nm, respectively. This Factor was studied at different time intervals to select the suitable time needed for microdetermination of DO.HCl and LD as shown in Fig. (3). During the first 30 minutes, the absorbance increases with increase of time. Approximately in the period 32 – 60 and 35 – 45 minutes for DO.HCl and LD, respectively, the absorbance is nearly constant and after that the time slowly increases. Therefore, the most suitable time for microdetermination of DO.HCl and LD using 4-AAP and universal buffer of pH = 12 is 32 and 35 minutes, respectively.

The temperature is one of the factors that highly affect the formation of coloured products. Figure (4) shows the effect of temperature on the coupling products between active ingredient (DO.HCl or LD) and 4-AAP in the temperature range from 15 to 60 ^{0}C. It was observed that by increasing the temperature from 15 to 30 ^{0}C and 15 ^{0}C to 35 ^{0}C in case of DO.HCl and LD with 4-AAP at pH = 12, respectively, the absorbance increases. In between 30-40 ^{0}C for DO.HCl and 35- 50 ^{0}C for LD, the absorbance is approximately constant. Above these temperatures, the lighting in colour of the coupling products is observed and the absorbances slowly decrease. Therefore, It is concluded that the most suitable temperature for microdetermination of DO.HCl and LD with 4-AAP using universal buffer of pH = 12 are 30 ± 2 and 40 ± 2 ^{0}C, respectively (Figure (4).

Under the optimum conditions, a correlation was obtained between absorbance (A) and the concentration (C) over the range 27.90-170.6 and 49.30-221.8 µg mL^{-1} of DO.HCl and LD, respectively (Fig. (5)). The apparent molar absorptivity, Sandell sensitivity, standard deviation (SD), and coefficient of variation (CV) for each active ingredient are tabulated in Table (2). The apparent molar absorpitivity (ε) of the resulting coloured products are found to be 3.375x10^4 and 2.434x10^4 L. mol^{-1}.cm^{-1}, whereas Sandell sensitivities are 3.9x10^{-3} and 2.9x10^{-3} g cm^{-2} for DO.HCl and LD, respectively. The correlation coefficient is found to be 0.999, while the SD is 0.04-0.22 and 0.05-0.10 for DO.HCl and LD, respectively. The low values of CV and SD indicate the high accuracy, precision, and reproducibility of the proposed method to determine the cited drugs.

Interference

This factor is studied depending on the nature of our active ingredient where it may be secreted naturally in body or present as pharmaceutical preparation. This study is concentrated on the additives, which may commonly present in the pharmaceutical preparations or natural secreted fluids in urine. The common tolerances, which examined with our active ingredients (DO.HCl and LD) using 4-AAP and universal buffer of pH 12 reagents, are glucose, acetone, urea, ascorbic acid, catechol, phenol, pyrogallol, resorcinol and hydroquinone. From Tables (3), it is observed that, the presence of tolerances such as glucose, acetone, urea, pyrogallol or hydroquinone with DO.HCl and glucose with LD as ten folds, have completely no effect on the microdetermination of LD as seen from the percent recoveries (99.40 – 100.0%). The presence of ascorbic acid in the media has a great effect on the quantitativeness of the reaction. As the ascorbic acid concentration decreases to have the same concentration of active ingredient, the microdetermination of DO.HCl or LD can be carried out approximately without interference (percent recovery = 102 – 107 %). The presence of phenol or catechol with DO.HCl or phenol, catechol, pyrogallol, resorcinol or hydroquinone with LD as ten folds, they highly affect the microdetermination of the active ingredients giving high % error. By decreasing the concentration of phenol or hydroquinone to be the same as DO.HCl or LD the percent recovery raised up to 97 or 106 %, respectively.

Finally, we can concluded that, quantitative determination of DO.HCl can be carried out in the presence of glucose, acetone, urea, hydroquinone, pyrogallol and low concentration of ascorbic acid and phenol. In case of LD, the quantitative determination can be applied in presence of glucose and low concentrations of ascorbic acid and phenol.

Determination of LD in LD-CD synthetic mixtures

In order to prove the applicability to determine LD in some pharmaceutical forms like Levocare, synthetic mixtures containing LD and CD were prepared and analyzed for LD determination using our proposed procedure and the data obtained are listed in Table (3). In these mixtures, the percent recovery of LD is ranged from 101.4 to 102.4 %. These data reflect the high sensitivity of this procedure for microdetermination of LD in presence of CD.

Application

Determination of LD in pharmaceutical forms

LD used as neurotrasmitter in the management of neural disorders such as those associated to Parkinson's disease [27]. LD formulation associated with CD, which enhances the action of LD by inhibiting its decarboxylation and lengthening its therapeutic effect as a result.

Quantitative determination of LD in pharmaceutical preparation (tablets), which has collected from the commercial markets, is carried out using the proposed spectrophotometric method and official HPLC method [28].

From HPLC method, the retention time was found to be = 2.12 min for LD and 4.10 min for CD. Then the proposed and HPLC methods are applied on the drug matrix which prepared before (see experimental part). The results obtained in Table (5) show that the percentage recovery is found to be very close to those obtained by the official method with percent error of 1.10-3.70% that can be neglected where it present in the international acceptable range of error for pharmaceuticals determination [29]. In order to check the confidence and correlation between the suggested procedure and the official method for microdetermination of LD, it is better to calculate the t- and F-tests (Table 5). The calculated t-test at the 95% confidence level is 0.91-1.02, while, the calculated F values are 4.0-5.85. It is obvious from these results that the t and F values did not exceed the theoretical values. The results obtained are considered to be of high accuracy and the methods can be successfully applied for the microdetermination of LD in row materials and in the commercial pharmaceutical preparations which contain LD.

Determination of DO.HCl in pharmaceutical preparations (ampoules)

The DO.HCl ampoule is used as an injection treatment with patients who suffering from ischemic heart disease. Determining DO.HCl in dosage form, manufactured in the local companies, is the mean idea of this paper in order to test the accuracy and presicion of the proposed procedure. The concentration of the drugs in the dosage forms is calculated from the appropriate calibration graph. Before carrying out microdetermination, the absorption spectrum of the drugs is carried out. It is found that, there is no shift in λ_{max} due to the presence of other constituents present in the dosage forms.

Table (5) shows the results obtained for the determination of DO.HCl in dosage forms coming from two different com-

panies and labeling mg per ampoule. Two different ampoule concentrations of DO.HCl; 76.61 and 153.23 mg/L; were determined applying the proposed procedure under selected optimum conditions of pH, time, temperature, ratio and wavelength and using official HPLC technique [30]. The data listed in Table (5) show the precision and reproducibility of the results with a percent error of 1.80–5.0% with SD = 0.11 - 0.22. The low values of SD indicate the successful application of our proposed method for the determination of DO.HCl in pharmaceutical preparations.

The results are compared with those obtained applying the official method [28]. The DO.HCl solution, using HPLC, is found to have a retention time of = 2.93 min. The results obtained are compared statistically by F- and t-tests with those obtained by official method on the sample of the same batch. The F- and t-test values obtained at the 95% confidence level are 4.0-6.84 and 0.85 – 0.17, respectively. It is clear from Table (5) that, the calculated F- and t-test values does not exceed the theoretical tabulated values indicating that there is no significant difference between accuracy of the proposed and the official methods.

The final recommendation is that the proposed procedure can be applied for microdetermination of DO.HCl in pharmaceutical preparations. The percent error can be neglected where the ampoule contain not less than 95.0% and not more than 105.0 % of the labeled amount of dopamine hydrochloride [29, 31].

Quantitative determination of pharmaceutical preparations using standard addition method (SAM)

The standard addition method (SAM) is applied to support the validity of application of our proposed procedure for microdetermination of DO.HCl and LD in their pharmaceutical preparations and to minimize the effect of any matrix interference's in quantitative determination of active ingredient in the commercial available drugs.

The SAM is applied on pharmaceutical preparations containing LD and DO.HCl as active ingredients. The extrapolation of the line of calibration curve intersected with the x-axis at 49.00 and 29.00-29.50 mg\L with percent error of 0.6 and 1.0% and SD = 0.22 and 1.07 for LD and DO.HCl, respectively (as shown in Table (6)). This gives the real concentration of active ingredient (LD and DO.HCl) present in the pharmaceutical preparations. The high values of the percent recovery reflect the high efficiency of applying standard addition method for microdetermination of the drugs under investigation in their pharmaceutical preparations.

CONCLUSION

The proposed method for DO.HCl and LD estimation is advantageous over many reported methods. This may be attributed to their sensitivity, rapidity, noninterference with other ingredients usually present in pharmaceutical preparations, precision and good agreement with the official method. Hence this method can be used for the routine analysis.

BIBLIOGRAPHY

1. Juan, C.; Yan-Ping, S. and Jing-Yan L., J. Chromatography A, 2003, 1003 (1-2), 127-132.
2. Jan S.; Grzegorz W.; Oliver F.; Andreas W. and Lothar W., Phytochemistry, 2001, 59 (2), 315-320.
3. Loutala, P., "Catechol O-methyltransferase: Glucuronidation of Inhibitors and Methylation of Substrates". University of Helsink, Finland, 2000.
4. Marino, F.; Cosentino, M.; Bombelli, R.; Ferrari, M.; Lecchini, S. and Frigo, G., Exp. Hematol., 1999, 27: 489-495.
5. Musso, N. R.; Brenci, S.; Setti, M.; Indiveri, F.; Lotti, G., J. Clin. Endocrinol. Metab., 1996, 81, 3553-3557.
6. Rodriguez, M. C.; Obeso, J. A. and Olanow, C. W., Ann. Neurol., 1998, 44, S 175.
7. Professor Philip G. Strange, http:/www.tocris. com/ dopamine. htm, published October 2000.
8. Kaakkola, S.; Tuomainen, P.; Wurtman, R. J. and Mannisto, P. T., J. Neural. Transm.; Parkinsons Dis. Dementia Sect., 1992, 4(2), 143.
9. Puttaswamy.; Jagadeesh, R.V. and Vaz, Nirmala., Central European Journal of Chemistry., 2005, 3 (2), 326-346.
10. Coello, J.; Maspoch, S. and Villegas, N., *Talanta*, 2000, ***53(3), 627-637***.
11. Nagaraja, P.; Vasantha, R.A. and Sunitha, K.R., J. Pharm. Biomed. Analysis, 2001, 25:3-4, 417-424.
12. Afkhami, A. and Khatami, H.A., J. Analytical. Chem., 2003, 58 (2), 135-138.
13. Nagaraja, P.; Murthy, K. C. Srinivasa; Yathirajan, H. S. and Mohan, B. M., Indian J. Pharm. Sci., 1998, 60(2), 99-101.
14. Nour El-Dien, F. A. and Rashwan, N. A. A., Egypt. J. Chem., 1995, 38(2), 125-39.
15. Zhang, Y.X.; Zhang, Z.J. and Yang, F. Chinese J. Chem. 2008, 26(3), 489-494.
16. Özkan, S.A. Chromatographia 2007, 66 (SUPPL. 1), S3-S13.
17. Wang, J.; Zhou, Y.; Liang, J.; He, P.G. and Fang, Y.Z. Chromatographia 2005, 66(5-6), 265-270.
18. Hou, S.; Zheng, N.; Feng, H.; Li, X. and Yuan, Z. Anal. Biochem., 2008, 381(2), 179-184.
19. Yao, H.; Sun, Y.Y.; Lin, X.; Cheng, J. and Huang, L. Luminescence 2006, 21(2), 112-117.
20. Önal, A.; Caglar, S. Chem. and Pharmac. Bull. 2007, 55(4), 629-631.
21. Abdulrahman, L.K.; Al-Abachi, A.M. and Al-Qaissy, M.H. Analytica Chimica Acta, 2005, 538(1-2), 331-335.
22. Shaikh, S.M.T.; Manjunatha, D.H.; Harikrishna, K.; Ramesh, K.C. and Kumar, R.S. J. Anal. Chem. 2008, 63(7), 637-642.
23. Nagaralli, B.S.; Seetharamappa, J.; Melwanki, M.B.; Ramesh, K.C. and Keshavayya, J. J. AOAC Internat., 2002, 85(6), 1288-1292.
24. Chen, Y.; Wang, S. and Zhou, T. Anal. Lett., 1998, 31, 1233.
25. Uslu, B. and Ozkan, S. Anal. Lett., 2002, 35, 303.
26. Nour El-Dien, F.A.; Zayed, M.A.; Mohamed, Gehad G. and El-Nahas, Reham G. J. Biomed. Biotech., 2005, 1, 1-9.
27. Bravo, E.L. Endcor Rev., 1994, 15, 356-68.
28. Sinclair, D.; Shenkin, A. and Lorimer, A.R. Ann. Clin. Biochem., 1991, 28, 417-9.
29. British Pharmacopiea, 1998,1150-1151.
30. HPLC, applications (Macherey-NAGel), K. Gobler, Dissertation, 1980, Erlangen-Numberg, Germany.
31. U.S. Pharmacopeia, National Formulory, 1995.

Table (1): *Effect of different pH on molar absorbitivity (∈) of DO.HCl and LD with 4-AAP using universal buffer (pH = 2-12).*

	λ_{max} (nm)	∈ (L mol^{-1} cm^{-1}) DO.HCl	∈ (L mol^{-1} cm^{-1}) LD
De-ionized water	**278**	**5.77x10^4**	**5.52x10^4**
pH = 2	260	5.25x10^4	4.89x10^4
3	260	5.04x10^4	4.87x10^4
4	260	5.02x10^4	5.23x10^4
5	279	5.6x10^4	5.60x10^4
6	279	6.03x10^4	5.92x10^4
7	179	6.28x10^4	5.88x10^4
8	279	6.37x10^4	5.88x10^4
9	279	6.54x10^4	5.84x10^4
10	279	6.49x10^4	5.84x10^4
11	282	7.10x10^4	5.88x10^4
12	285	8.31x10^4	7.37x10^4
	475	1.46x10^4	----------
	454	----------	1.05x10^4

Table (2): *Different analytical parameters for the determination of DO.HCl and LD using 4-AAP reagent and universal buffer (pH = 12).*

Parameters	DO.HCl	LD
λ_{max} (nm)	475	454
T (°C)	30 ± 2	40 ± 2
t, min	32	35
Detection range (mg/L)	37.90 – 170.6	49.30 – 221.8
Correlation coefficient	0.999	0.999
ε (L.mol^{-1}cm^{-1})	3.375x10^4	2.434x10^4
SD	0.04-0.22	0.05-0.15
CV (%)	0.06-0.16	0.02-0.10
Sandell Sensitivity (μg cm^{-2})	3.9x10^{-3}	2.9x10^{-3}

Table (3): *Effect of different tolerances on the determination of DO.HCl and LD using 4-AAP and universal buffer of pH = 12.*

Tolerant	DO.HCl			LD		
	Fold	A	% recovery	Fold	A	% recovery
Drug	-----	0.333	100.0	-----	0.414	100.0
Glucose	10	0.333	100.0	10 1	0.416 0414	100.5 100.0
Acetone	10	0.331	99.40	-----	-----	-----
Ascorbic acid	10 1 0.5	0.005 0.341 0.331	------- 102.4 99.40	10 1 0.5	0.017 0.447 0.418	4.10 107.9 101.1
Urea	10 1 0.5	0.327 0.330 0.331	98.23 99.10 99.40	-------	------	------
Catechol	10 0.5	0.766 0.522	230.0 156.7	10 1	0.66 0.547	159.4 132.1
Phenol	10 1 0.5	0.310 0.323 0.328	93.1 97.00 98.50	10 1 0.5	0 .455 0.433 0.412	109.9 104.6 99.50
Pyrogallol	10 1	0.328 0.306	98.50 91.90	10 1	0.643 0.551	155.3 133.1
Resorcinol	10 1	0.040 0.095	------- -------	10 1	0.328 0.352	79.20 85.00
Hydroquinone	10 1	0.329 0.331	98.80 99.40	10 1 0.5	0.306 0.442 0.395	73.90 106.7 95.40

Table (4): *Determination of LD in the LD-CD synthetic mixture using 4-AAP and universal buffer. [4-AAP] = $3x10^{-3}$ M, [LD] = $2.44x10^{-3}$ M, [CD] = $2.4x10^{-4}$ M, pH = 12, t = 35 min, T = 38 ^{0}C, λ_{max} = 454 nm.*

[LD] = 96.23 mg\L			[LD] = 192.5 mg/L		
A	(mg/L) found	% recovery	A	(mg/L) found	% recovery
0.300	96.75	100.5	0.539	196.33	102
0.304	98.42	102.3	0.544	197.42	102.5
0.300	96.75	100.5	0.541	197.16	102.4
0.305	98.83	102.7	0.543	198.0	102.8
0.303	98.00	101.8	0.547	197.66	102.7
A_{mean} 0.302	97.58	101.4	A_{mean} 0.541	197.52	102.46
SD	0.29		SD	0.36	
CV %	0.29		CV %	0.18	

Table (5): *Determination of DO.HCl and LD in pharmaceutical preparations using 4-AAP reagent and universal buffer (pH = 12).*

Drug	Name of preparation	[Drug], Taken (mg/l)	Proposed method			Officila method			t-test*	F-test *
			Found (mg/l)	% recovery	SD	Found (mg/l)	% recovery	SD		
DO.HCl	- Dopamine Fresenius	76.61	76.62	100.4	0.11	77.23	100.8	0.28	0.85	6.48
		153.2	153.7	100.3	0.13	152.4	99.17	0.26	1.40	4.00
	- Dopamine Pierre Fabre	76.61	79.28	103.4	0.12	78.30	102.2	0.22	1.43	3.36
		153.2	151.2	98.60	0.16	154.9	101.1	0.25	1.70	2.44
LD	- Alpha Chem. Advanced Pharmaceutical Industries Co. (ACAPI).	98.60	97.00	98.20	0.13	98.20	99.60	0.26	0.91	4.00
		197.2	191.9	97.30	0.11	188.8	98.40	0.26	1.02	5.58

F- test: are the values for V as degree of freedom for variation at 95% confidence level = 6.39.*
t-test: are the values for V as degree of freedom for 95% confidence level = 2.779.*

Drug	Name of preparation	[Drug], Taken (mg/l)	Proposed method		
			Found (mg/l)	% recovery	SD
DO.HCl	- Dopamine Fresenius	28.70	29.00	101.0	0.15
	- Dopamine Pierre Fabre	28.70	29.50	105.0	0.12
LD	- Alpha Chem. Advanced Pharmaceutical Industries Co. (ACAPI).	49.30	49.00	99.40	0.22

Table (6): *Determination of DO.HCl and LD in pharmaceutical preparations using standard addition method.*

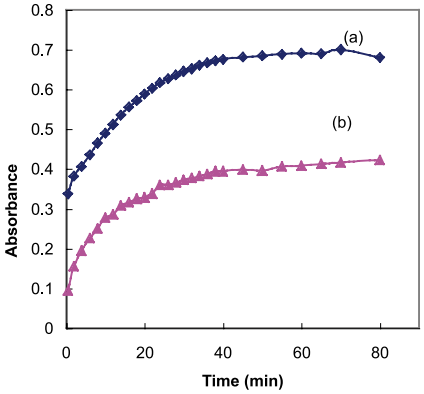


Fig. (3): *Effect of time on the absorbance of (a) DO.HCl and (b) LD with 4-AAP and universal buffer of pH = 12*

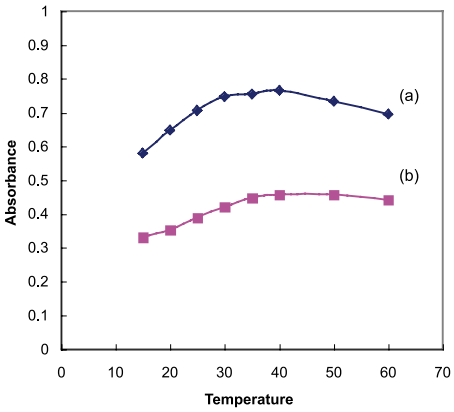


Fig. (4): *Effect of temperature on the reaction of (a) DO.HCl and (b) LD with 4-AAP using universal buffer of pH = 12*

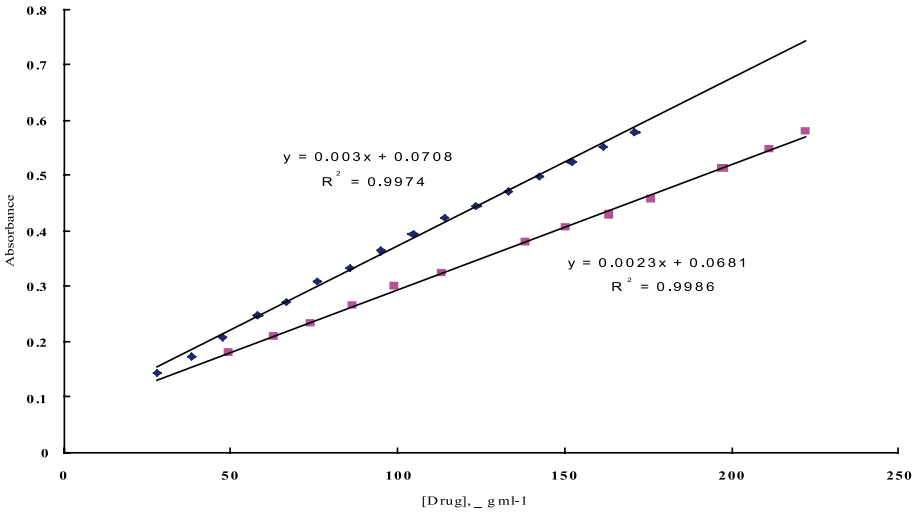


Fig. (5). *Calibration curves for the determination of (a) DO.HCl (pH = 12, t = 32 min, T = 30 °C and λ = 475 nm) and (b) LD (pH = 12, t = 38 min, T = 38 °C and λ = 454 nm) using 4-AAP reagent.*