www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

Novel analytical approach for reducing the consumption of organic solvents in the charge transfer-based spectrophotometric analysis of losartan potassium

Ibrahim A. Darwish[a]*, Ashraf M. Mahmoud[b], Abdul-Rahman A. Al-Majed[a]

[a]Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451.
[b]Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt

ABSTRACT

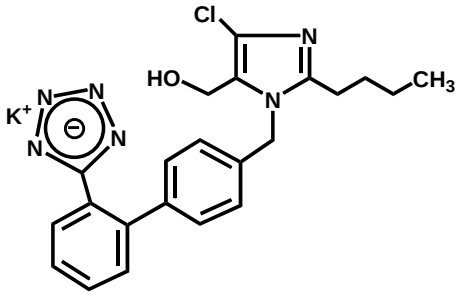
The present study describes the development of a novel analytical approach that can reduce the consumption of organic solvents in the charge transfer (CT)-based spectrophotometric analysis of losartan potassium (LOS) by 50-fold. The proposed approach employed 96-microwell assay plates for carrying out the reaction. In this approach, the CT reaction between LOS and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) as a π-electron acceptor was performed in the microwells (200-µl of organic solvent). The color signals of the CT complex were measured at 460 nm by microwell-plate reader. The optimum conditions for the proposed approach were established and the analytical procedures were recommended. The proposed approach offered high sensitivity and precision; the limits of detection and quantitation were 2.47 and 7.49 µg ml^{-1}, respectively. The proposed approach was successfully applied to the analysis of pharmaceutical dosage forms that contain LOS with good accuracy and precision, and the results were compared favorably with a reference method. The approach described herein has great practical value in the routine analysis of LOS in quality control laboratories, as it offers the reduction in the exposures of the analysts to the toxic effects of organic solvents, reduction in the analysis cost by 50-fold, and it has a high throughput property. Although the approach was validated for LOS, however, the same methodology could be used for any electron-donating analyte for which a CT reaction can be performed.

Keywords: Charge-transfer reaction; Organic solvents; Spectrophotometry; High throughput analysis; Losartan potassium.

1. INTRODUCTION

Losartan potassium (LOS); 2-butyl-4-chloro-1-[p-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl-imidazole-5-methanol potassium, is a new non-peptide angiotensin II receptor antagonist with antihypertensive activity, due mainly to selective blockade on AT_1 receptors and the consequent reduced pressor effect of angiotensin II (Martindale, 2002). LOS is used in the management of hypertension with a lower incidence of side effects such as cough, which develops with typical angiotensin-converting enzyme inhibitors. It can be used alone or combined with the diuretic hydrochlorothiazide (HCT) in patients with moderate heart failure (Reid, 2001)

The therapeutic importance of LOS was behind the growing interest in the development of many methods for its analysis in bulk drug, dosage forms, and/or biological fluids. These methods include high-performance liquid chromatography (Hertzog, 2002), thin-layer chromatography (McCarthy, 1998), gas chromatography (Maurer, 1998), capillary electrophoresis (Gonzalez, 2002), and radioreceptor assay (Soldner, 1998). These methods are not simple to perform, time-consuming, and/or utilize expensive instruments that are not available in most quality control laboratories.



Losartan potassium (LOS)

Spectrophotometry is the most widely used technique in pharmaceutical analysis because of its inherent simplicity and wide availability in most quality control laboratories (Darwish, 2007; Darwish, 2008; Darwish, 2009, Darwish, 2010). The molecular interactions between electron-donating pharmaceutical compounds and electron acceptors are generally associated with

* Corresponding Author
Email: idarwish@ksu.edu.sa
Contact: +966-14677348 Fax: +966-14676220
Received on: 03-02-2010
Revised on: 17-09-2010
Accepted on: 19-09-2010

the formation of intensely colored charge-transfer (CT) complexes, which absorb radiation in the visible region. The rapid formation of these CT complexes leads to their widespread utility in the development of simple and convenient spectrophotometric methods for many pharmaceutical compounds (Bebawy, 1999; Darwish and Refaat, 2006; Khashaba, 2000; Saleh, 2003). In a previous study (Darwish, 2005), the electron-donating capability of LOS has been proved, and CT-based spectrophotometric methods for determination of LOS in its dosage forms have been described. However, these methods suffered a major drawback which was the consumption of large volumes of organic solvents. This leads to high analysis cost, and more importantly, the incidence of exposure of the analysts to these toxic solvents.

Many studies supported the positive association between the exposure to laboratory work with organic solvents and occurrence of dose-related neurotoxic symptoms (Fidler, 1987), increased risk of some reproductive outcomes (especially preterm and posterm births) among the women working with certain laboratory tasks (Wennborg, 2002), increased risk of spontaneous abortion among pregnant women (Lindbohm, 2007; Wennborg, 2000), and increased risk of lymphohaemalopoietic cancer (leukemia and lymphomas) in both men and women (Kristensen, 2008). Reduction of human exposure to organic solvents is one of the main objectives of hygienists, public authorities, World Health Organization, environment protection agencies, and occupational safety and health administrations. For these reasons, investigating new alternative methodological approaches to reduce the consumption of organic solvents in CT-based spectrophotometric analysis is very important.

The present study describes the development of a novel approach that can reduce the consumption of organic solvents in the CT-based spectrophotometric analysis of LOS by 50-fold. In this approach, the reaction was carried out in 96-microwell plates (200-µl volume) instead of the conventional volumetric flasks (10,000-µl volume). The color signals were measured by microwell-plate reader. The analytical approach described herein offered some additional advantages: (1) reduction in the exposures of the analysts to the toxic effects of organic solvents, (2) reduction in the analysis cost by 50-folds, and (3) providing a high throughput analytical methodology that can facilitate the processing of large number of samples in a relatively short time. This property was attributed to the use of multi-channel pipettes for rapid and efficient dispensing the solutions, carrying out the analytical reaction in 96-well plates (as reaction vessels), and measuring the color signals in the 96 wells at ~ 30 seconds by the plate reader.

2. EXPERIMENTAL

2.1. Apparatus

Microwell-plate reader (ELx 808 IU, Bio-Tek Instruments Inc. Winooski, USA). Microwell plates were a product of Corning/Costar Inc. (Cambridge, USA). Finnpipette adjustable 8 channel-pipette (Sigma Chemical Co. St. Louis., USA).

2.2. Chemicals and dosage forms

LOS (Hetero Drugs Ltd., Hyderabad, India) was obtained and used as working standard. DDQ (Merck, Schuchardt, Munich, Germany) was 0.25% (w/v) prepared fresh daily in methanol. Losartan® tablets (Amriya Pharmaceutical Industries, Alexandria, Egypt) are labeled to contain 50 mg LOS per tablet. Lozapress H® tablets (Sigma Pharmaceutical Industries, Egypt) are labeled to contain 50 mg LOS and 12.5 mg HCT per tablet.

2.3. Preparation of standard and tablets sample solutions

2.3.1. Preparation of stock standard solutions

Into a 5-ml calibrated flask, 10 mg of LOS was accurately weighed, dissolved in 2 ml methanol, and completed to volume with the same solvent. This stock solution was diluted with methanol to obtain concentrations in the range of 16-240 µg ml^{-1}.

2.3.2. Preparation of tablets sample solutions

Twenty tablets were weighed and finely powdered. A quantity of the powder equivalent to 20 mg LOS was transferred into a 10-ml calibrated flask, dissolved in 2 ml methanol, swirled and sonicated for 5 min, completed to volume with the methanol, shaken well for 15 min, and filtered. The first portion of the filtrate was rejected, and a measured volume of the filtrate was diluted quantitatively with methanol to concentrations range of 16-240 µg ml^{-1}.

2.5. General analytical procedure

One hundred microliters of the standard or sample solution (16–240 µg ml^{-1}) was transferred into wells of microwell-plates. One hundred microliters of DDQ solution (0.25%, w/v) was added, and the reaction was allowed to proceed at room temperature (25±5 ◦C) for 10 min. The absorbances of the resulting solutions were measured at 460 nm by the microwell-plate reader. Blank wells were treated similarly except 100 µl of methanol was used instead of sample, and the absorbances of the blank wells were abstracted from those of the other wells.

3. RESULTS AND DISCUSSION

3.1. Strategy for development of the proposed analytical approach

The present study was directed to the development of a novel approach that can reduce the consumption of

organic solvents in the CT-based spectrophotometric analysis of LOS. LOS was selected was based on its therapeutic importance, its proven electron-donating capability, and the urgent need to overcome the major drawback of the previously reported CT-based spectrophotometric analysis of LOS, which is the consumption of large volumes of organic solvents (Darwish, 2005). Previous studies for the CT reactions of polyhalo-/polycyanoquinones electron acceptors revealed that DDQ was one of the most reactive reagents, as its CT reaction proceeds rapidly, and yielded high sensitive assays when compared with other polyhaloquinones (Bebawy, 1999; Darwish, 2005; Khashaba, 2000). For these reasons, DDQ was selected as electron acceptor in the development of the approach described herein.

The following sections describe the establishment of the optimization factors that influence the chemical reaction and the analytical performance of the proposed approach.

3.2. Optimization of the analytical conditions

The interaction of LOS with DDQ, and the spectral characteristics of the formed chromogen, and reaction mechanism have been demonstrated in a previous study (Darwish, 2005). The optimization of experimental conditions affecting the reaction in the 96-well format was investigated by altering each reaction variable in a turn while keeping the others constant. In all cases, measurements were carried out at 460 nm, as this maximum gave the highest absorptivities and ultimately the highest analytical sensitivity. The results of variations in the DDQ concentrations indicated that 100 µl of 0.25% (w/v) was the optimum DDQ concentration, as this concentration gave the highest absorbances. Previous studies (Darwish, 2005; Khashaba, 2000) demonstrated that the interaction of electron-donors with DDQ in polar solvents (e.g. acetonitrile and methanol) produces CT complexes with molar absorptivity values higher than those produced in non-polar solvents (e.g. chloroform). Methanol was selected for the subsequent experiments because it offered high sensitivity. The optimum reaction time was determined by monitoring the color development in the microwells at room temperature (25±5°C). Complete color development was attained after 10 min. The developed colors remained stable at room temperature for at least a further 30 min.

3.4. Validation of the proposed methodology

3.4.1. Linearity and sensitivity

Under the above mentioned optimum reaction conditions, the calibration curve for the analysis of LOS by the proposed analytical approach was constructed by plotting the absorbances as a function of the corresponding concentrations. The regression equations for the results were derived using the least-squares method. Beer's law plot (n = 5) was linear with very small intercept (–0.0517) and good correlation coefficient (0.9978) in a general concentration range of 8 – 120 µg ml^{-1}. The limits of detection (LOD) and limits of quantitation (LOQ) were determined (USP, 2008) using the formula: LOD or LOQ = κSDa/b, where κ = 3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope. The LOD and LOQ values were 2.47 and 7.49 µg ml^{-1}, respectively.

3.4.2. Accuracy and precision

Accuracy of the proposed methodology was assessed by the standard addition method. Known amount (30 mg) of LOS was added to pre-determined drug-containing dosage forms, and then determined by the recommended procedure of the proposed approach. The mean analytical recovery (calculated as the ratio between the concentrations measured to the concentrations taken for analysis, expressed as percentages) was found to be 101.01 ± 0.63% indicating the accuracy of the proposed methodology.

The precisions of the proposed methodology were determined on samples of LOS solutions at three concentration levels (10, 60, and 120 µg ml^{-1}) by analyzing 5 replicates of each sample as a batch in a single assay run. The relative standard deviations (RSD) were 0.87, 0.63, and 0.74% at the concentration levels of 10, 60, and 120 µg ml^{-1}, respectively. This data provided the high precision of the methodology for the routine application in quality control laboratories. This high level of precision was attributed to the accuracy of the volumes that have been concomitantly dispensed in the microwells by multi-channel pipettes, and completeness of the reaction the small volume (200 µl).

3.5. Application of the proposed approach in the analysis of dosage forms

Two commercially available pharmaceutical dosage forms for LOS were subjected to the analysis by the proposed and reported methods (Dawish, 2005), and

Table 1: Analysis of LOS in its tablets by the proposed and reported methods

Tablet	Recovery (% ± SD)		*t*-test [b]	*F*-test [b]
	Proposed method	Reported method [a]		
Lozapress H®	99.79±1.02	100.52±1.30	0.99	1.63
Losartan®	100.98±1.31	101.66±1.51	0.76	1.34

[a] Darwish, 2005.

[b] The tabulated values of t and F at 95% confidence limit are 2.78 and 6.39, respectively.

the obtained results were then statistically compared with each other. The mean percentage recoveries, relative to the labeled amounts, obtained by the proposed procedures were 100.98 ± 1.31 and 99.79 ± 1.02% (Table 1). In the t- and F-tests, no significant differences were found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level. This indicated similar accuracy and precision of both the proposed and reported methods.

4. CONCLUSIONS

The present study described the development and validation of a novel analytical approach for reducing the consumption of organic solvents in the spectrophotometric analysis based on CT reactions by 50-fold. In this approach, the reaction was carried out in 96-microwell plates (200-µl reaction volume) instead of the conventional volumetric flasks (10,000-µl volume). The color signals were measured by microwell-plate reader instead of the conventional spectrophotometer. The analytical approach described herein has the following advantages:

- Reduction in the consumption of organic solvents in the CT-based spectrophotometric analysis of LOS, accordingly reduction in the exposures of the analysts to the toxic effects of organic solvents.
- Reduction in the analysis cost by 50-folds which can be reflected on the price for the finished dosage forms, thus it can reduce the expenses for the medications to the patients.
- Providing a high throughput analytical methodology that can facilitate the processing of large number of samples in a relatively short time. This property was attributed to the use of multichannel pipettes for efficient dispensing the solutions, carrying out the analytical reaction in 96-well plates (as reaction vessels), and measuring the color signals in the 96 wells at ~ 30 seconds by the plate reader.
- Although the approach was developed and validated for the analysis of the antihypertensive drug LOS, however, it is also anticipated that the same methodology could be used for essentially any analyte that can exhibit electron-donating capability.

ACKNOWLEDGMENT

The authors thank the Research Center at College of Pharmacy, King Saud University for funding the work (grant No. C.P.R.C. 225).

REFERENCES

Bebawy LI, El-Kousy NM, Saddik JK, Shokry M. Spectrophotometric determination of fluoxetine and sertraline using chloranil, 2,3 dichloro-5,6-dicyano- benzoquinone and iodine. J Pharm Biomed Anal 1999, 21: 133-142.

Darwish IA, Abdel-Rahman SA, Mahmoud AM, Hassan AI. Spectrophotometric determination of H_2-receptor antagonists via their oxidation with cerium (IV). Spectrochim Acta 2008, 69: 33-40.

Darwish IA, Abdel-Rahman SA, Mahmoud AM, Hassan AI. Sensitive indirect spectrophotometric method for determination of H_2-receptor antagonists in pharmaceutical formulations. Intl J Biomed Sci 2007, 3: 124-131.

Darwish IA, Abdine HH, Amer SM, Al-Rayes LI. Spectrophotometric study for the reaction of fluvoxamine 1,2-naphthoquinone-4-sulphonate: kinetic, mechanism, and use for determination of fluvoxamine in its dosage forms. Spectrochim Acta A 2009, 72: 897-902.

Darwish IA, Refaat IH. Spectrophotometric analysis of selective serotonin reuptake inhibitors based on formation of charge-transfer complexes with tetracyanoquinondimethane and chloranilic acid. J AOAC Intl 2006, 89: 326-333.

Darwish IA, Sultan MA, Al-Arfaj HA. Selective kinetic spectrophotometric method for determination of gatifloxacin based on formation of its *N*-vinylchlorobenzoquinone derivative. Spectrochim Acta A 2010, 75: 334-339.

Darwish IA. Analytical study for the charge transfer complexes of losartan potassium. Anal Chim Acta 2005, 549: 212-220.

Fidler AT, Baker EL, Letz RE. Neurobehavioural effects of occupational exposure to organic solvents among construction painters. Br J Indust Med 1987, 44: 292-308.

Gonzalez L, Akesolo U, Jimenez RM, Alonso RM. Application of capillary zone electrophoresis to the screening of some angiotensin II receptor antagonists. Electrophoresis 2002, 23: 223-229.

Hertzog DL, McCafferty JF, Fang X, Tyrrell RJ, Reed RA. Development and validation of a stability-indicating HPLC method for the simultaneous determination of losartan potassium, hydrochlorothiazide, and their degradation products. J Pharm Biomed Anal 2002, 30: 747-760.

Khashaba PY, El-Shabouri SR, Emara KM, Mohamed AM. Analysis of some antifungal drugs by spectrophotometric and spectrofluorimetric methods in different pharmaceutical dosage forms. J Pharm Biomed Anal 2000, 22: 363-376.

Kristensen P, Hilt B, Svendsen K, Grimsrud TK. Incidence of lymphohaematopoietic cancer at university laboratory: a cluster investigation. Eur J Epidemiol 2008, 23: 11-15.

Lindbohm ML, Taskinen HT, Sallman M, Hemminki K. Spontaneous abortions among women exposed to organic solvents. Am J Indust Med 2007, 17: 449-463.

Martindale. The Complete Drug Reference. 33th edition, London, Pharmaceutical Press, 2002, pp. 921-922.

Maurer HH, Kraemer T, Arlt JW. Screening for the detection of angiotensin-converting enzyme inhibitors, their metabolites, and AT II receptor antagonists Ther Drug Monit 1998, 20: 706-713.

McCarthy KE, Wang G, Tsai EW, Gilbert RE, Ip DP, Brooks MA. Determination of losartan and its degradates in COZAAR® tablets by reversed-phase high-performance thin-layer chromatography. J Pharm Biomed Anal 1998, 17: 671-677.

Reid IA. In: Basic & Clinical Pharmacology. 8th edition, Beirut, Librairie du Liban, 2001, pp. 292-298.

Saleh GA, Askal HF, Darwish IA, El-Shorbagi AA. Spectroscopic analytical study for the charge-transfer complexation of certain cephalosporins with chloranilic acid. Anal Sci 2003, 19: 281-287.

Soldner A, Spahn-Langguth H, Palm D, Mutschler E. A radioreceptor assay for the analysis of AT1-receptor antagonists: Correlation with complementary LC data reveals a potential contribution of active metabolites. J Pharm Biomed Anal 1998, 17: 111-124.

USP, The United States Pharmacopeia 31, The National Formulary 26, Rockville, US Pharmacopeial Convention Inc, 2008, pp. 683-686.

Wennborg H, Bonde JP, Stenbeck M, Olsen J. Adverse reproduction outcomes among employee in biomedical research laboratories. Scand J Work Environ Health 2002, 28: 5-11.

Wennborg H, Lennart B, Harri V, Gösta A. Pregnancy outcome of personnel in swedish biomedical research laboratories. J Occup Environ Med 2000, 42: 438-446.