

## NEW SPECTROFLUORIMETRIC METHODS FOR THE SIMULTANEOUS DETERMINATION OF OLMESARTAN MEDOXAMIL AND AMLODIPINE BESYLATE IN THEIR COMBINED TABLETS

H.W. DARWISH<sup>a,b</sup>, A.H. BAKHEIT<sup>a</sup>, I.A. DARWISH<sup>a\*</sup>

<sup>a</sup>*Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia.*

<sup>b</sup>*Department of Analytical Chemistry, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, ET 11562, Cairo, Egypt.*

Olmесartan medoxamil (OLM, an angiotensin II receptor blocker) and amlodipine besylate (AML, a dihydropyridine calcium channel blocker), are co-formulated in a single-dose combination for the treatment of hypertensive patients whose blood pressure is not adequately controlled on either component monotherapy. In this work, two simple, sensitive, and reliable spectrofluorimetric methods have been developed and validated for the simultaneous determination of OLM and AML in their combined tablets. These methods are first derivative and ratio subtraction methods for OLM and AML, respectively. The optimum assay conditions were established and the methods were validated with respect to linearity, sensitivity, accuracy, precision and specificity. Regression analysis showed excellent correlation between the peak amplitude or fluorescence intensity with the concentration over the concentration ranges of 0.08-1 and 0.25-2  $\mu\text{g mL}^{-1}$  for OLM and AML, respectively. The proposed methods were successfully applied for the assay of the two drugs in their laboratory prepared mixtures and combined pharmaceutical tablets with recoveries not less than 99.0%. No interference was observed from common pharmaceutical additives. The results were favourably compared with those obtained by a reference method.

(Received April 24, 2012; Accepted October 12, 2012)

*Keywords:* Olmesartan medoxamil; Amlodipine besylate; Spectrofluorimetry; Simultaneous determination; Pharmaceutical tablets.

### 1. Introduction

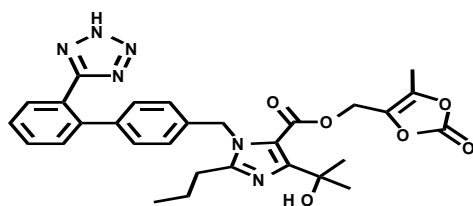
Olmесartan medoxamil (OLM, Fig. 1) is chemically known as (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy-4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)-phenyl]phenyl}methylimidazol-5-carboxylate. It is a potent and selective angiotensin AT1 receptor blocker. (K. Koga, S. Yamagishi et al. 2002) It has been approved for the treatment of hypertension in the United States, Japan and European countries. The drug contains a medoxamil ester moiety which is cleaved rapidly by an endogenous esterase to release the active olmesartan. (L.R. Schwocho and H.N. Masonson 2001) There are various methods for analysis of OLM alone or in combination with other drugs. These methods include spectrophotometry,<sup>3-9</sup> spectrofluorimetry,<sup>9</sup> HPTLC,<sup>10</sup> Mass,<sup>11</sup> LC-MS-MS,<sup>12</sup> CZE,<sup>13</sup> and HPLC<sup>14,15</sup>.

Amlodipine besylate (AML, Fig. 1) is chemically known as 3-ethyl-5-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methylpyridine-3,5-dicarboxylate benzene sulphonate. It is a dihydropyridine calcium channel blocker used in the treatment of hypertension and angina pectoris.<sup>16</sup> AML is official in the British Pharmacopoeia (BP) which describes HPLC for its assay in the bulk powder.<sup>17</sup> Several analytical methods have been reported for the determination of AML in pharmaceutical formulations and/or biological fluids. These methods

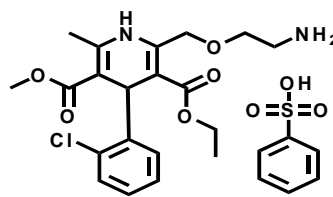
---

\* Corresponding author: idarwish@ksu.edu.sa

include spectrophotometry,<sup>18-20</sup> spectrofluorimetry,<sup>21-24</sup> anodic stripping voltammetry,<sup>25-26</sup> HPLC,<sup>27-32</sup> HPTLC,<sup>33</sup> capillary electrophoresis<sup>34</sup> and micellar electrokinetic chromatography.<sup>35</sup>



**Olmesartan medoxomil (OLM)**



**Amlodipine besylate (AML)**

*Fig. 1. Chemical structures of olmesartan medoxomil (OLM) and amlodipine besylate (AML).*

Recently, OLM has been marketed in combination with AML in tablet dosage form (Olmesar® tablets). The oral administration of this combination has been proved to be more effective than either of the two drugs in a single-drug therapy for treatment of hypertension.<sup>36</sup> Few methods are available for the simultaneous analysis of OLM and AML combination. These methods include spectrophotometry,<sup>36-40</sup> HPLC<sup>38,41,42</sup> and TLC.<sup>42</sup> These methods suffered from lower sensitivity and selectivity (e.g. UV-based spectrophotometry), employed intensive instrumentation (e.g. HPLC) or need laborious manipulation (e.g. TLC). Spectrofluorimetric technique is characterized by its inherent high sensitivity, improved selectivity, practical simplicity, and wide availability of in quality control laboratories. However, to the best of our knowledge, based on extensive literature survey, no attempt has yet been made to employ spectrofluorimetry for the simultaneous determination of OLM and AML. Therefore, the aim of this work was directed to the development of simple, sensitive and selective spectrofluorimetric methods for the simultaneous determination of OLM and AML in their combined dosage form.

## 2. Experimental

### Apparatus

Fluorescence measurements were carried out on a RF-1501 version 3.0 spectrofluorimeter (Shimadzu Corporation Kyoto, Japan) equipped with a 150 W xenon lamp and 1 cm quartz cells. The slit widths of both the excitation and emission monochromators were set at 1.5 nm. The calibration and linearity of the instrument were frequently checked with standard quinine sulphate ( $0.01 \mu\text{g mL}^{-1}$ ). Wavelength calibration was performed by measuring  $\lambda_{\text{excitation}}$  at 275 nm and  $\lambda_{\text{emission}}$  at 430 nm; no variation in the wavelength was observed.

### Materials

OLM was obtained from AK Scientific Inc. (CA, USA). AML was obtained from Pfizer Inc. (New York, USA). The purities of OLM and AML were 99.5 %. Olmesar® tablets (Macleods Pharmaceutical Ltd., Mumbai, India) labeled to contain 5 mg of AML and 20 mg of OLM (Batch No: PM00058803). Double distilled water was obtained through WSC-85 water purification system (Hamilton Laboratory Glass Ltd., KY, USA) and used throughout the work. All solvents and materials used throughout this study were of analytical grade.

### Preparation of OLM and AML standard solutions

Stock solutions of OLM ( $160 \mu\text{g mL}^{-1}$ ) and AML ( $200 \mu\text{g mL}^{-1}$ ) were prepared by dissolving 16 mg and 20 mg of OLM and AML, respectively in 100 mL methanol. Appropriate volumes of these stock solutions were diluted to give working solutions of 8 and  $40 \mu\text{g mL}^{-1}$  for OLM and AML, respectively. Stock and working solutions were stable for at least two weeks when stored refrigerated at 4 °C.

### Preparation of pharmaceutical tablets sample solutions

Twenty Olmesar<sup>®</sup> tablets were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 40 mg of OLM and 10 mg of AML was extracted into methanol with the aid of shaking and the methanolic extract was filtered. The filtrate was diluted with methanol to obtain final concentrations of 200 and 50  $\mu\text{g mL}^{-1}$  for OLM and AML, respectively. Aliquots of Olmesar<sup>®</sup> tablet solution were diluted with 0.1 M HCl to obtain final concentrations within the specified range of the assay, and the samples were subjected to the analysis according to the general recommended procedure.

### General recommendation procedure and construction of calibration curves

Aliquots of standard working solutions equivalent to 0.08-1 and 0.25-2  $\mu\text{g mL}^{-1}$  of OLM and AML, respectively were accurately transferred into two separate series of 5-mL volumetric flasks, and completed to volume with 0.1 M HCl. The emission spectra of the prepared standard solutions were recorded from 220 to 600 nm using  $\lambda_{\text{excitation}}$  at 251 nm and stored in the computer.

**First derivative spectral method for OLM.** For the determination of OLM in presence of AML, the first derivative of the stored emission spectra of OLM ( $\lambda_{\text{excitation}}$  at 251 nm) were computed with  $\Delta\lambda = 10$  nm. The amplitude of the first derivative peak of OLM was measured at 378.0 nm. The calibration graph was constructed by relating the peak amplitudes at 378.0 nm to the corresponding OLM concentrations and the regression equation for the analysis was derived.

**Ratio subtraction spectral method for AML.** The calibration curve was constructed relating the fluorescence intensity of zero order emission spectra of AML at 455 nm ( $\lambda_{\text{excitation}}$  at 251 nm) to the corresponding concentrations and the regression equation for the data is computed.

### Assay of laboratory prepared mixtures

Aliquots of the standard working solutions of OLM and AML were transferred into a series of 5-mL volumetric flasks, completed to volume with 0.1 M HCl and mixed well. For determination of OLM, the procedures under 'First derivative spectral method for OLM' were applied. For determination of AML, the emission spectra (fluorescence intensity at each wavelength) of the laboratory prepared mixtures were divided by the spectrum of 0.5  $\mu\text{g mL}^{-1}$  of OLM (divisor). The fluorescence intensity in the plateau region was subtracted at wavelength from 350- 400 nm (the constant). The obtained curves were multiplied (fluorescence intensity at each wavelength) by the spectrum of 0.5  $\mu\text{g mL}^{-1}$  of OLM. Then the fluorescence intensities for the ratio subtraction of the laboratory prepared mixtures were measured at 455 nm for determination of AML.

## 3. Results and discussion

Olmesar<sup>®</sup> tablets are combined dosage form containing the angiotensin II receptor blocker OLM and the calcium channel blocker AML. It has been used in the treatment of hypertension. This study was designed to develop simple and accurate methods for the simultaneous determination of OLM and AML in Olmesar<sup>®</sup> tablets. Because of the inherent high sensitivity, improved selectivity, practical simplicity, and wide availability of in quality control laboratories high sensitivity and simplicity of spectrofluorimetry, it was attempted in this study.

### Spectral characteristics and optimization of assay conditions

Both of OLM and AML exhibited native fluorescence with  $\lambda_{\text{emission}}$  at 417 and 455 nm for OLM and AML, respectively, after excitation of both drugs at 251 nm (Fig. 2).

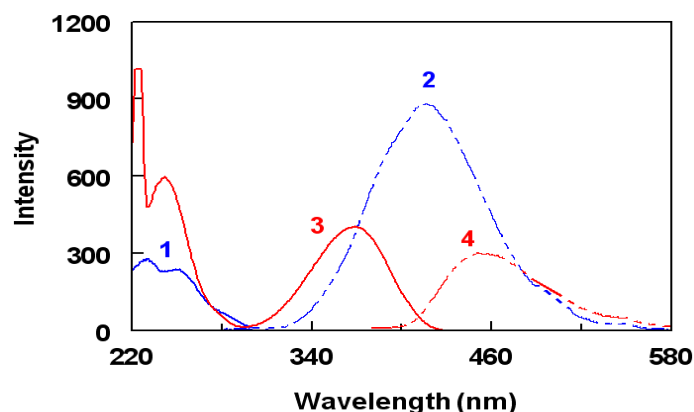


Fig. 2. Excitation (solid lines) and emission (dashed lines) for OLM (1 for excitation and 2 for emission) and AML (3 for excitation and 4 for emission). Concentrations of both OLM and AML were  $1 \mu\text{g mL}^{-1}$  in  $0.1 \text{ M HCl}$ .

The conditions for the zero order emission spectra of both OLM and AML were optimized. The fluorescence characteristics of OLM and AML were investigated in several solvent media (water, methanol,  $0.1 \text{ M HCl}$  and  $0.1 \text{ M NaOH}$ ). The selection of the diluting solvent was based on the sensitivity of measurement and stability of the fluorescence readings. For OLM, fluorescence intensity in  $0.1 \text{ M HCl}$  was higher than those obtained in water by at least five-folds, while the maximum fluorescence intensity of AML was obtained in  $0.1 \text{ M HCl}$  and water (Fig. 3).

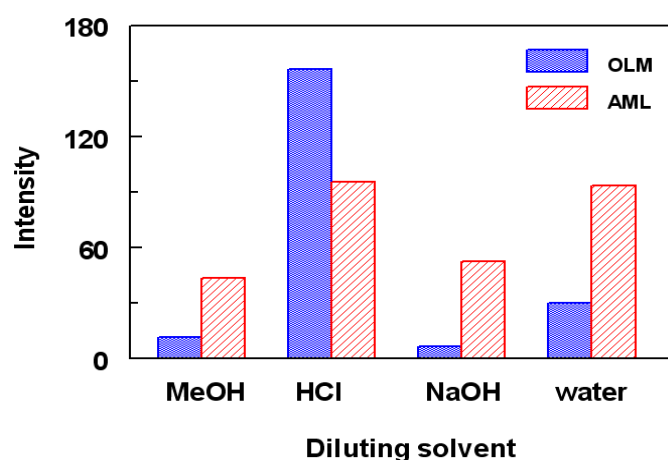


Fig 3. Effect of dilution solvents on the fluorescence intensity of OLM ( $0.16 \mu\text{g mL}^{-1}$ ) and AML ( $2 \mu\text{g mL}^{-1}$ ). Concentration of HCl and NaOH was  $0.1 \text{ M}$ .

Stability of readings in case of OLM was minimum in neutral or basic medium. This was attributed to its hydrolysis (as a prodrug) to the olmesartan moiety that did not exhibit fluorescence.<sup>9</sup> The stability of OLM and AML solutions (in  $0.1 \text{ M HCl}$  and water for OLM and AML, respectively) was followed by measuring the fluorescence intensities at 15-min intervals. Fluorescence intensity values were stable for at least 1 h. Under these optimized conditions, the emission spectra of OLM and AML after their excitation at  $251 \text{ nm}$  are given in Fig. 4A. Obviously, the emission spectra of both OLM and AML were overlapped. This fact limited the direct determination of OLM and AML in presence of each other. Therefore the first derivative and ratio subtraction fluorescence spectroscopic techniques were applied for the simultaneous determination of OLM and AML in their combined dosage form.

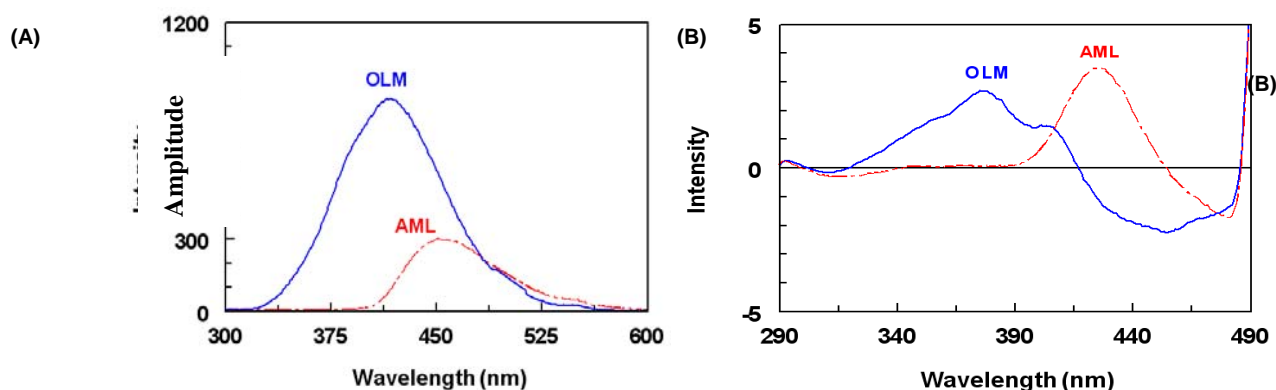


Fig. 4. Zero order of emission (A) and first derivative of ratio (B) spectra of OLM and AML. In zero order emission spectra, concentrations of OLM and AML were  $0.5 \mu\text{g mL}^{-1}$ . In first order emission spectra, concentrations of OLM and AML were  $0.16$  and  $1.5 \mu\text{g mL}^{-1}$ , respectively.

### Methods development

Derivative spectrometry is a very useful analytical technique for determining the binary and multicomponent mixtures of drugs with overlapped spectra. In this work, first derivative spectrofluorimetric method was reported to accomplish the determination of OLM in its binary mixture with AML without prior chemical separation. The first derivative curves of both OLM and AML ( $\Delta\lambda = 10 \text{ nm}$ ) were computed (Fig. 4B). It was obvious that OLM could be determined solely at  $378 \text{ nm}$  where AML response was zero. Linear relationship was obtained upon plotting the peak amplitudes of the first derivative spectra at  $378 \text{ nm}$  against their corresponding concentrations of OLM (Fig. 5B).

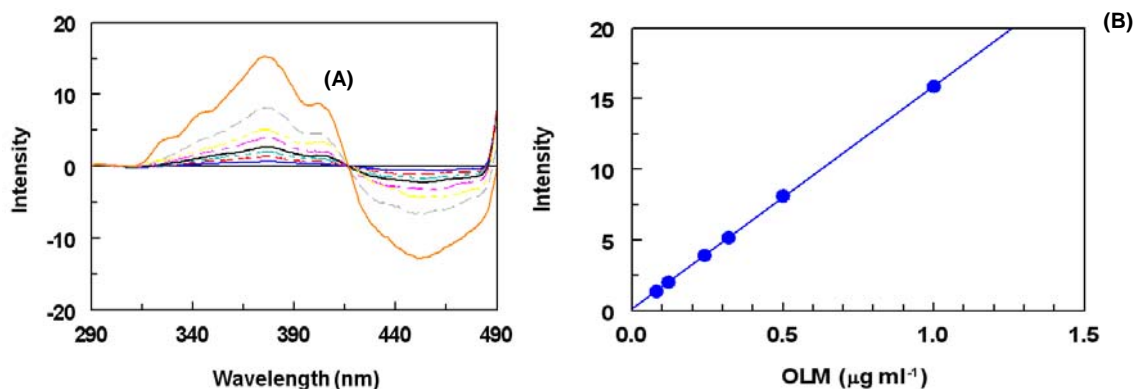


Fig. 5. Calibration spectra (A) and calibration line (B) for determination of OLM by first derivative spectral method. Concentrations of OLM were  $0.08\text{--}1 \mu\text{g mL}^{-1}$  in  $0.1 \text{ M HCl}$ . Values presented in set B were average of three determinations.

For determination of AML, ratio subtraction method was applied.<sup>43</sup> Zero order emission spectra of the standard solutions of AML were recorded, and the linearity between the fluorescence intensities and the corresponding concentration of AML was checked at the selected wavelength ( $455 \text{ nm}$ ). The method depended on the following: when a mixture of AML (X) and OLM (Y) where the spectrum of (Y) was more extended (Fig. 4A), the determination of (X) could be done by scanning the zero order emission spectra of the laboratory-prepared mixtures (AML and OLM), dividing them by carefully chosen concentration ( $0.5 \mu\text{g mL}^{-1}$ ) of standard OLM ( $Y' = \text{divisor}$ ) producing a new ratio spectra that represent  $(X/Y') + \text{constant}$ , then subtraction of the fluorescence

intensities of these constants ( $Y/Y'$ ) in plateau region (350-400 nm) as shown in Fig. 6A. This step was followed by multiplying the obtained spectra by the divisor ( $Y'$ ) to ultimately obtain, the original spectra of (X) which were used for direct determination of AML at 455 nm and calculation of the concentration from the corresponding regression equation. A linear correlation was obtained between the fluorescence intensities (FI) and the corresponding concentration of AML ( $C_2$ ) at 455 nm (Fig. 7).

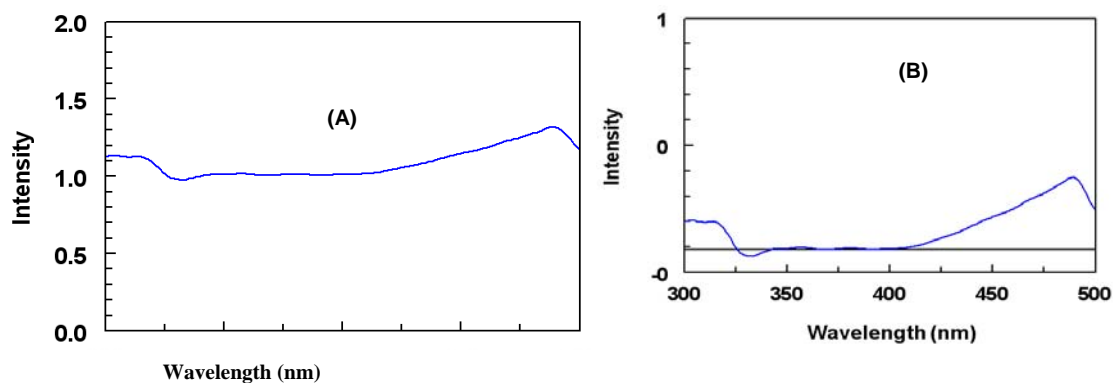


Fig. 6. (A) Spectrum of laboratory-made mixture of OLM and AML (1:1) divided by the spectrum of OLM ( $0.5 \mu\text{g mL}^{-1}$ ). (B) the same spectrum after subtraction of fluorescence intensity value of OLM from the original spectrum.

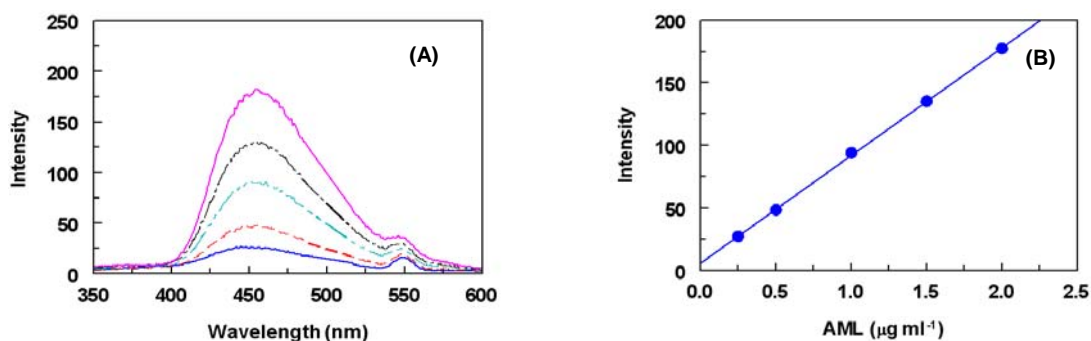


Fig. 7. Zero order emission spectra (A) and calibration line (B) for determination of AML by ratio subtraction spectral method. Concentrations of AML were  $0.25\text{--}2 \mu\text{g mL}^{-1}$ . Values presented in set B were average of three determinations.

### Methods validation

The proposed methods were validated according to the ICH-guidelines for validation of the analytical procedures<sup>44</sup> in terms of the linearity, sensitivity, accuracy, specificity, repeatability and reproducibility.

**Linearity and sensitivity.** A linear correlation was obtained between peak amplitude or fluorescence intensity and the corresponding concentrations of OLM and AML in the ranges of 0.08-1 and  $0.25\text{--}2 \mu\text{g mL}^{-1}$ , respectively. The regression equations were:

$$\text{For OLM: PA} = 15.76 C_1 + 0.1482 \quad (r = 0.9999)$$

$$\text{For AML: FI} = 86.155 C_2 + 6.0476 \quad (r = 0.9997)$$

where PA was the peak amplitude of the first order spectrum of OLM at 378 nm, FI was fluorescence intensity of the zero order spectrum of AML at 455 nm,  $C_1$  and  $C_2$  were the concentrations of OLM and AML in  $\mu\text{g mL}^{-1}$ , respectively, and  $r$  was the correlation coefficient. LOD and LOQ were calculated<sup>44</sup> according to the following equations:

$$\text{LOD} = 3.3 \sigma / S \text{ and } \text{LOQ} = 10 \sigma / S$$

Where,  $\sigma$  was the standard deviation of the intercept of regression line and  $S$  was the slope of regression line of the calibration curve. The results are given in Table 1.

Table 1. Validation report of the proposed spectrofluorimetric methods for determination of OLM and AML

Parameters	First derivative method for OLM	Ratio subtraction method for AML
Linear range ( $\mu\text{g mL}^{-1}$ )	0.08–1.0	0.25–2.0
Intercept $\pm$ SD	0.15 $\pm$ 0.037	6.05 $\pm$ 1.098
Slope $\pm$ SD	15.76 $\pm$ 0.076	86.16 $\pm$ 0.893
Correlation Coefficient	0.9999	0.9997
LOD ( $\mu\text{g mL}^{-1}$ )	0.008	0.04
LOQ ( $\mu\text{g mL}^{-1}$ )	0.02	0.13
Accuracy <sup>a</sup>	101.18 $\pm$ 0.98	99.61 $\pm$ 1.67
Repeatability <sup>a</sup>	101.32 $\pm$ 1.15	99.99 $\pm$ 1.02
Intermediate precision <sup>a</sup>	100.90 $\pm$ 1.54	100.37 $\pm$ 2.61

<sup>a</sup> corresponding values are average of three determinations  $\pm$  SD.

**Accuracy.** The accuracy of the proposed methods was tested by analyzing triplicate samples of OLM and AML solutions. The recovery percentages were 101.18  $\pm$  0.98 and 99.61  $\pm$  1.67% for OLM and AML, respectively (Table 1). These results revealed the excellent accuracy of the proposed methods. The results obtained by applying the proposed methods for simultaneous determination of OLM and AML in bulk forms and in dosage form (Olmesar<sup>®</sup> tablets) were statistically compared with those results obtained by the reference method.<sup>37</sup> It was concluded that with 95% confidence, there was no significant difference between the proposed and reference methods in terms of their accuracy and precision as the calculated  $t$ - and  $F$ - values were less than the theoretical values<sup>45</sup> (Table 2).

Table 2. Analysis of OLM and AML in their bulk drugs and Olmesar<sup>®</sup> tablets by the proposed spectrofluorimetric and reference methods

Method	Recovery (% $\pm$ SD) <sup>a</sup>	$t$ -value <sup>b</sup>	$F$ -value <sup>b</sup>
Bulk drug			
First derivative method for OLM	101.18 $\pm$ 0.98 (6)	1.06 (2.23)	3.02 (5.05)
Ratio subtraction method for AML	99.61 $\pm$ 1.67 (5)	0.38 (2.31)	3.45 (6.39)
Reference method <sup>c</sup>			
For OLM	100.33 $\pm$ 1.71 (6)		
For AML	99.93 $\pm$ 0.90 (5)		
Olmesar <sup>®</sup> tablets			
First derivative method for OLM	100.54 $\pm$ 0.75 (5)	1.71 (2.31)	2.024 (6.39)
Ratio subtraction method for AML	100.04 $\pm$ 1.19 (5)	0.97 (2.31)	1.668 (6.39)
Reference method <sup>c</sup>			
For OLM	99.53 $\pm$ 1.07 (5)		
For AML	99.38 $\pm$ 0.92 (5)		

<sup>a</sup> Figures in parentheses are the number of determinations.

<sup>b</sup> Figures in parentheses are theoretical values for  $t$ - and  $F$ - at confidence level of 95%.

<sup>c</sup> Reference 37.



**Repeatability and reproducibility.** Intra-assay precision was assessed by analyzing varying concentrations of OLM (0.08, 0.36 and 0.5  $\mu\text{g mL}^{-1}$ ) and AML (0.25, 0.5 and 1.5  $\mu\text{g mL}^{-1}$ ) in triplicate in one assay batch. The inter-assay precision was assessed by analyzing the same concentrations in triplicate on 3 consecutive days. The average recovery percentages were around 100% and the low relative standard deviations (RSD) indicated the high accuracy and precision of the proposed methods, respectively (Table 1).

**Specificity.** OLM and AML were determined in laboratory prepared mixtures containing different ratios of the two drugs. The good recovery % and low standard deviations (SD) proved the high specificity of the proposed methods (Table 3).

*Table 3. Determination of OLM and AML in laboratory-prepared mixtures by the proposed spectrofluorimetric methods*

Taken conc. ( $\mu\text{g mL}^{-1}$ )		Recovery (%)	
OLM	AML	Ratio subtraction method for OLM	First derivative method for AML
0.50	0.50	97.35	99.18
0.50	0.25	97.93	99.80
0.75	0.25	100.53	101.20
1.00	0.25	98.56	97.06
0.25	0.50	99.26	101.30
Mean		98.73	99.71
SD		1.24	1.74

Specificity was also investigated by observing any possible interferences from the common tablet excipients, such as lactose, gelatin, magnesium stearate and starch. It was found that these excipients did not interfere with the proposed methods as indicated from the obtained good recovery values (Table 2).

#### 4. Conclusions

In this study, direct, simple, and sensitive spectrofluorimetric procedures were developed and validated for the simultaneous determination of the two antihypertensive drugs; OLM and AML in their combined tablets. The proposed methods combine the rapidness and simplicity advantages of traditional spectrometric methods together with other important analytical merits, such as sensitivity and specificity. The proposed methods did not require elaborate treatment or sophisticated experimental set-up that are associated with the existing methods. Moreover, simplicity was illustrated by the minimum requirement of chemicals and solvents since methanol was the only organic solvent used in the procedure, and final measurement of both drugs was done in aqueous acidic media. Consequently, the proposed methods were cost-effective and environmentally friendly. The validation of the proposed methods according to the ICH guidelines proved the applicability and great value of these methods for routine application in quality control laboratories for the simultaneous analysis of drug combination tablets without prior separation or excipient interference.

#### Acknowledgement

The author thanks the Deanship of Scientific Research and the Research Center of the College of Pharmacy, King Saud University for supporting this study.



## References

- [1] K. Koga, S. Yamagishi, M. Takeuchi, Y. Inagaki, S. Amano, *Mol. Med.* **8**, 591(2002).
- [2] L.R. Schwocho, H.N. Masonson, *J. Clin. Pharmacol.* **41**,515(2001).
- [3] M. Celebier, S. Altinoz, *Pharmazie* **64**, 419(2007).
- [4] T. Sharmal, S.C. Si, D. Gowrishankar, *J. Pharm. Res.* **3**, 1553(2010).
- [5] P. Mehulkumar, P. Ramesh, V.V. Kumar, R. Srinivas, P.V. Diwan, *Pharm. Meth.* **2**, 36(2011).
- [6] S.S. Kadukara, P.N. Ranjanea, S.S. Ranhera, S.V. Gandhia, *Pharm. Rev.* 161(2008).
- [7] M. Celebier, S. Altinoz, Hacettepe Univ. *J. Fac. Pharm.* **27**, 119 (2007).
- [8] N.J. Shah, B.N. Suhagia, R.R. Shah, N.M. Patel, *Indian J. Pharm. Sci.* **69**, 834 (2007).
- [9] M. Farouk, O. Abd EL-Aziz, A. Hemdan, M. Shehata, *J. Am. Sci.* **7**, 300 (2011).
- [10] N.J. Shah, B.N. Suhagia, *Indian J. Pharm. Sci.* **69**, 834 (2007).
- [11] L. Dongyang, H. Pei, N. Matsushima, *J. Chromatogr. B* **856**, 190 (2007).
- [12] V.V. Vaidya, M.N. Shikha, *Chromatographia* **67**, 147 (2008).
- [13] M. Celebier, A. Sacide, *Chromatographia* **66**, 929 (2007).
- [14] C.V. Patel, A.P. Khandhar, *Eurasian J. Anal. Chem.* **2**, 159 (2007).
- [15] T. Murakami, H. Konno, N. Fukutsu, *J. Pharm. Biomed. Anal.* **1**, 7 (2008).
- [16] S.C. Sweetman, *Martindale-The Complete Drug Reference*, 36th edn. The Pharmaceutical Press, London, 1214, 1420, 1421 (2009).
- [17] *The British Pharmacopoeia*, Her Majesty's Stationery Office, London, 138 (2010).
- [18] N. Rahman, M. Singh, M. N. Hoda, *Il Farmaco* **59**, 913(2004).
- [19] A.K. Muthu, T.R. Gupta, S. Sharma, A.A. Smith, R. Manavalan, N. Kannappan, *Int. J. Chem. Sci.* **6**, 2233 (2008).
- [20] C.S. Saxena, S.C. Chturvedi, *Asian J. Chem.* **21**, 4118 (2009).
- [21] A.A. Gazy, *Bull. Fac. Pharm. Cairo Univ.* **41**, 245 (2003).
- [22] H.M. Abdel-Wadood, N.A. Mohamed, A.M. Mahmoud, *Spectrochim. Acta A* **70A**, 564 (2008).
- [23] L.E. Abdel Fattah, T.A. Mohamed, E.A. Taha, *J. Drug Res.* **29**, 83(2008).
- [24] R.A. Shaalan, T.S. Belal, *Drug Test. Anal.* **2**, 489 (2010).
- [25] A.A. Gazy, *Talanta* **62**, 575(2004).
- [26] M. Kazemipour, M. Ansari, A. Mohammadi, H. Beitollahi, R. Ahmadi, *J. Anal. Chem.* **64**, 65 (2009).
- [27] V.G. Dongre, S.B. Shah, P.P. Karmuse, M. Phadke, V.K. Jadhav, *J. Pharm. Biomed. Anal.* **46**, 583 (2008).
- [28] S.J. Joshi, P.A. Karbhari, S.I. Bhoir, *Chromatographia* **70**, 557 (2009).
- [29] G. Bahrami, S. Mirzaeei, *J. Pharm. Biomed. Anal.* **36**, 163 (2004).
- [30] A.B. Baranda, R.M. Jimenez, R.M. Alonso, *J. Chromatogr. A* **1031**, 275 (2004).
- [31] P. Massaroti, L.A.B. Moraes, M.A.M. Marchioretto, N.M. Cassiano, G. Bernasconi, S.A. Calafatti, F.A.P. Barros, E.C. Meurer, J. Pedrazzoli, *Anal. Bioanal. Chem.* **382**, 1049 (2005).
- [32] Q. Zou, Y. Zhan, Z. Ge, P. Wei, P. Ouyang, *Arzneimittel Forschung* **59**, 383 (2009).
- [33] R. Kakde, N. Bawane, *J. Planar Chromatogr. Modern TLC* **22**, 115 (2009).
- [34] A.R. Fakhari, S. Nojavan, S. Haghgoo, A. Mohammadi, *Electrophoresis* **29**, 4583 (2008).
- [35] V. Martinez, J.A. Lopez, R.M. Alonso, R.M. Jimenez, *J. Chromatogr. A* **836**, 189 (1999).
- [36] P. Mehulkumar, Ramesh, K.V. Vinay, R. Srinivas, P.V. Diwan, *Asian J. Res. Chem.* **2**, 127 (2009).
- [37] T. Sharma, N. Mishra, S.C. Si, D.G. Shankar, *Der Pharmacia Lett.* **2**, 302 (2010).
- [38] S.B. Wankhede, S.B. Wadkar, K.C. Raka, S.S. Chitlange. *Indian J. Pharm. Sci.* **71**, 563 (2009).
- [39] P.S. Patil, N.D. Chivate, S.S. Shinde, H.N. More, S.A. Pishwikar, *Int. J. Chem. Tech. Res.* **3**, 267 (2011).
- [40] P.S. Patil, P.D. Kulkarni, M.S. Burkul, H.N. More, S.A. Pishawikar, *Int. J. Pharm. Tech. Res.* **3**, 668 (2011).
- [41] P.S. Patil, H.N. More, S.A. Pishawikar, *Int. J. Pharma. Pharmac. Sci.* **3**, 146 (2011).
- [42] Y. K. Asmita, V.M. Mahadeo, D.K. Laxman, R.D. Sunil, *Anal. Lett.* **43**, 251 (2010).
- [43] M.G. El-Bardicy, H.M. Lotfy, M.A. El-Sayed, M.F. El-Tarras, *J. AOAC Int.* **91**, 299(2008).

- [44] ICH, Validation of Analytical procedures: Methodology (Q2AR1), International Conference on Harmonization, Food and Drug Administration, USA, November 1996 and November 2005.
- [45] J. D. Hinchey, Practical statistics for chemical Research, 1<sup>st</sup> ed., London(1969).