

# Electro-oxidation and simultaneous determination of amlodipine and atorvastatin in commercial tablets using carbon nanotube modified electrode

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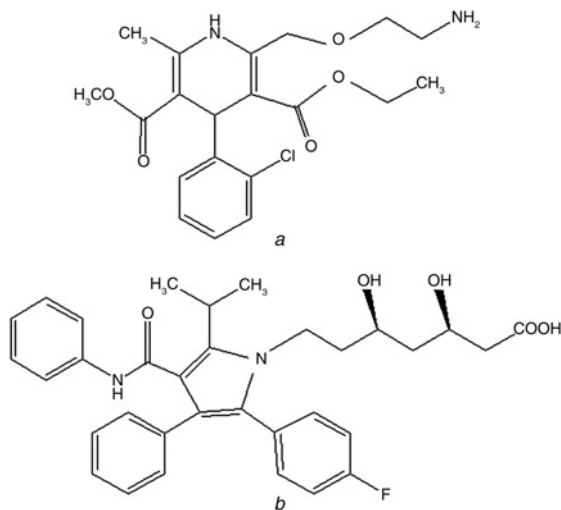
Amlodipine besylate and atorvastatin calcium have been determined by the simultaneous voltammetric method at a multi-walled carbon nanotubes:graphite (MWCNTs:G) paste electrode. In comparison with a glassy carbon electrode, the prepared electrode showed an increase in the peak current because of the high electroactive surface area and excellent electronic conductivity of MWCNTs. The dependence of currents and potentials on pH were investigated for these components at the surface of the MWCNTs:G paste electrode. Differential pulse voltammetry was applied as a sensitive technique for simultaneous determination of the drugs in commercial tablets. By anodic differential pulse voltammetry, the calibration plot was linear in the range of 2.5–100 µg/ml with standard deviation between 2.7–7.1 and 1.8–8.3% for amlodipine and atorvastatin, respectively. The detection limit was 1 µg/ml at the prepared electrode in the buffered solution pH 6.

**1. Introduction:** Amlodipine (AML, Fig. 1) is a dihydropyridine derivative with calcium antagonist activity. It is used in the management of hypertension, chronic stable angina pectoris and Prinzmetal variant angina [1]. Atorvastatin (ATOR, Fig. 1) is a selective and competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme (HMG-CoA) reductase. It is used to reduce low-density lipoprotein-cholesterol, apolipoprotein B and triglycerides and to increase high-density lipoprotein-cholesterol in the treatment of hyperlipidaemias [2, 3]. These drugs have been developed and commercialised to treat high blood pressure and high cholesterol in one dosage form.

Much literature has been published on non-electrochemical methods for determination of either AML [4] or ATOR [5–8]

alone or in combination with other drugs in pharmaceutical dosage forms or individually in biological fluids. In addition, numerous non-electrochemical methods have been reported for simultaneous determination of both AML and ATOR in solid oral dosage forms [9]. These methods are complicated, expensive and time-consuming compared with voltammetric techniques. Some electro-analytical methods including square-wave anodic stripping voltammetry on gold electrode [10], voltammetric determination using single- and multi-walled carbon nanotubes (MWCNTs) modified edge plane pyrolytic graphite electrodes [11], adsorptive square-wave anodic stripping voltammetry on a carbon paste electrode [12] and adsorptive square-wave anodic stripping voltammetry on a glassy carbon electrode [13] have been investigated for determination of AML in pharmaceutical dosage forms or in biological fluids. Moreover, ATOR has been determined by a few electroanalytical methods [14, 15] in pharmaceutical or in biological samples. There is a dearth of electroanalytical methods reporting the simultaneous determination of these compounds. To our knowledge, only Dogan-Topal *et al.* [16] have described the first derivative of ratio voltammetric methods for the determination of AML and ATOR in tablets in the presence of other substances. The ratio derivative method involves calculating and plotting one of the mathematical derivatives of the curve that offers an alternative approach for drug analysis. This technique depends on measuring the first derivative of the ratio voltammograms as a function of analyte concentration; whereas, differential pulse and square-wave voltammetric methods depend on the first derivative of the ratio-voltammetry measured at the selected potentials for AML and ATOR. The linear response was within the range of  $4 \times 10^{-6}$ – $1 \times 10^{-4}$  M for AML and  $2 \times 10^{-6}$ – $1 \times 10^{-4}$  M for ATOR.

The oxidation and reduction of most pharmaceutical compounds on bare electrodes have slow electron-transfer kinetics. The role of nanomaterials as a constituent of the electrode could assist electron transfer between the analyte and the electrode surface [17]. Various nanomaterials because of their unique properties have attracted great interest in numerous fields [18–22].



**Figure 1** Structural formulas of  
a AML  
b ATOR

The subtle electronic properties suggest that carbon nanotubes have the ability to promote electron-transfer reactions [23–25]. Therefore they can increase the sensitivity and selectivity of the analysis. In this Letter, we describe a simple, rapid and sensitive procedure for the simultaneous determination of AML and ATOR using a MWCNTs:G paste electrode.

## 2. Experimental

**2.1. Chemicals and materials:** AML 5 mg and ATOR 10 mg tablets were purchased from local pharmacies. MWCNTs (purity >96%) were purchased from Neutrino Co. The MWCNTs had an outer wall diameter distribution of <10 nm, a length of between 5 and 15  $\mu\text{m}$  and a special surface area of 180–190  $\text{m}^2/\text{g}$ . First, 0.04 M Britton-Robinson buffer solution was prepared in each of acetic acid, orthophosphoric acid and boric acid. Then, the solutions with varying pH were prepared by adding 0.2 M solution hydroxide. All aqueous solutions were prepared with doubly distilled deionised water. Analytical grade phosphoric acid, acetic acid, boric acid, sodium hydroxide, paraffin oil (density 0.84–0.89) and graphite powder (<50  $\mu\text{m}$ ) (all from Merck) were prepared.

**2.2. Instruments:** All the voltammetric measurements were performed with three electrode cells [26, 27]. A carbon paste electrode was used as a working electrode, saturated Ag/AgCl and Pt wire as reference and auxiliary electrodes, respectively. Transmission electron microscopy studies were performed by a Phillips transmission electron microscope. A digital pH meter was used for preparation of the buffer solution. Voltammetric experiments were performed using a  $\mu$ -AUTOLAB TYPE (III). An ultrasonic model STARSONIC 60 was used for cleaning the surface of the electrode.

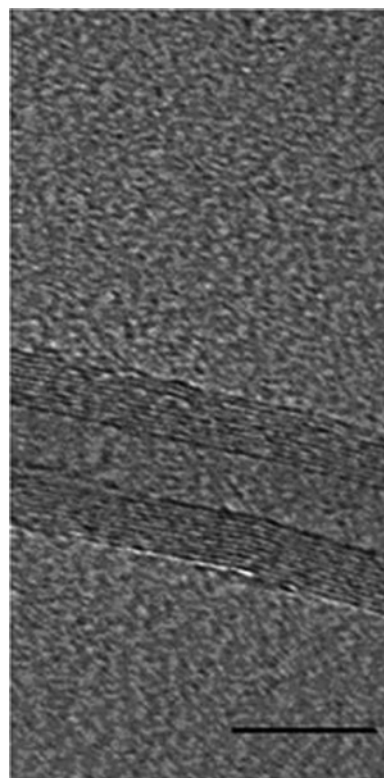
**2.3. Preparation of MWCNTs:G paste electrode:** For purification of MWCNTs, they were refluxed in the mixture of concentrated  $\text{H}_2\text{SO}_4$  and HCl (50:50 V/V) for 2 h, then washed with doubly distilled water and dried in an oven at 140°C for 24 h. Then, 20 mg MWCNTs were mixed with 20 mg graphite powder and 10  $\mu\text{l}$  of melted paraffin was added to the MWCNTs:G powder mixture. The mixture was homogenised in a mortar for 1 h. A portion of the composite mixture was packed into the end of a polytetrafluoroethylene tube. The electrical contact was made by forcing an Ag rod ( $r=1\text{ mm}$ ) down into the tube and into the back of the composite. The modified electrode surface was cleaned after each run by immersion into bare hexane normal and ethanol solutions. Then, it was sonicated to desorb the adsorbed material.

**2.4. Tablet assay procedure:** For determination of AML and ATOR in dosage forms, 20 tablets of AML (5 mg tablets) and 20 tablets of ATOR (10 mg tablets) were weighed, crushed and mixed together using a mortar and pestle for 25 min. A portion of powder equivalent to the weight of one tablet of each dosage form was accurately moved into a 200 ml volumetric flask containing 150 ml of Britton-Robinson buffer (pH 6). Then, the volumetric flask was sonicated for 20 min to dissolve AML or ATOR completely and the solution was then diluted with buffer solution to reach a starting concentration of 25 and 50  $\mu\text{g}/\text{ml}$  of AML and ATOR, respectively. A suitable aliquot of the solution was filtered through a 0.45  $\mu\text{m}$  nylon filter and was then transferred into the voltammetric cell.

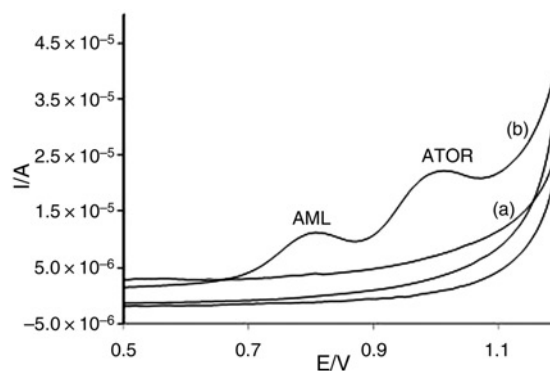
**2.5. Preparation of stock and standard solutions:** Stock solutions of atorvastatin calcium and amlodipine besylate (equivalent to about 200  $\mu\text{g}/\text{ml}$  of the free base) were prepared in Britton-Robinson buffer (pH 7). The stock solutions were protected from light using aluminium foil. Aliquots of the ATOR and AML stock solutions were transferred into 20 ml volumetric flasks. Later on, Britton-Robinson buffer (pH 6) were used to prepare solutions with final concentrations of 2.5, 5, 10, 25, 50, 75 and 100  $\mu\text{g}/\text{ml}$

for ATOR (maintaining the AML concentration at a constant level of 25  $\mu\text{g}/\text{ml}$ ) and concentrations of 2.5, 5, 10, 25, 50, 75 and 100  $\mu\text{g}/\text{ml}$  for AML (maintaining the ATOR concentration at a constant level of 25  $\mu\text{g}/\text{ml}$ ). Standard solutions of AML (20  $\mu\text{g}/\text{ml}$ ), ATOR (20  $\mu\text{g}/\text{ml}$ ) and a mixture of both drugs (20  $\mu\text{g}/\text{ml}$ ) were prepared for investigation of the electrochemical behaviour of AML and ATOR. All standard solutions were used freshly.

**3. Results and discussion:** In this study, the electrochemical behaviours of AML and ATOR were determined on the glassy carbon (GC) and MWCNTs:G paste electrode using cyclic and differential pulse voltammetric techniques. Fig. 2 presents the structure of the MWCNT pipe clearly. Fig. 3 shows the electro-oxidation behaviour of AML and ATOR at the (a) GC electrode and (b) MWCNTs:G paste electrode. The results showed very weak electro-oxidation behaviour at the GC



**Figure 2** TEM image of MWCNTs  
Scale bar = 5 nm



**Figure 3** Cyclic voltammograms of 20  $\mu\text{g}/\text{ml}$  AML and ATOR on surface of (a) bare GC electrode and (b) MWCNT:G paste electrode in buffer solution (pH 6.0)  
Scan rate: 100 mV/s

electrode. However, on the surface of the MWCNTs:G paste electrode a well-defined oxidation peak was obtained within the studied potential window.

**3.1. Effect of pH:** The influence of pH on the electrochemical behaviour of AML and ATOR in mixed solutions of them were examined by differential pulse voltammetry. In this investigation, voltammetric studies were performed in the pH range of 3–7 using 0.04 mol/l buffer solution as a supporting electrolyte. The results are shown in Fig. 4. Differential pulse voltammetric measurements showed an oxidation peak for each compound in all studied pH values. According to the results in Fig. 4, the peaks' separation and current have the maximum values at pH 6 for both drugs in mixture solutions. According to parts (a) and (b) of Fig. 4,  $E_{pa}$  of both drugs have linear relationship with the pH of the buffer solution regarding following equations

$$\begin{aligned} \text{AML: } E_{pa}(\text{mV}) &= -38.7 \text{ pH} + 990.3 \quad (R^2 = 0.979) \\ \text{ATOR: } E_{pa}(\text{mV}) &= -29.7 \text{ pH} + 1116.9 \quad (R^2 = 0.997) \end{aligned}$$

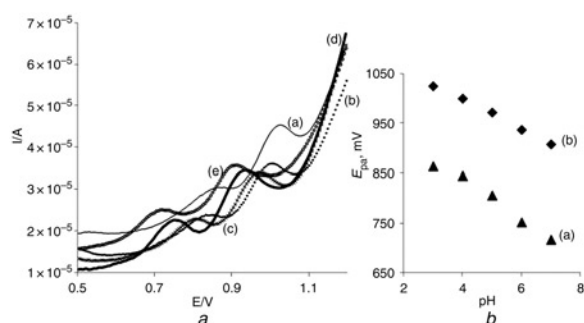
A value of about  $-39$  and  $-30$  mV per pH unit clearly indicates that two electrons and a proton are involved in the electro-oxidation on the surface of the carbon paste electrode.

**3.2. Effect of scan rates:** Useful information involving the electrochemical mechanism usually can be acquired from the relationship between peak current and scan rate. Therefore, the electrochemical behaviour of AML and ATOR simultaneously were investigated at different scan rates from 40 to 400 mV/s by cyclic voltammetry at the MWCNTs:G paste electrode. Although the potential scan rate increased, the positive shift in the potential peak occurred. This positive shift confirmed the irreversibility of the oxidation process. Also, only an anodic peak was observed with no associated cathodic peak in the reverse scan, which indicated that AML and ATOR oxidation on the MWCNTs:G paste electrode is an irreversible process. Fig. 5 shows CVs of 20  $\mu\text{g/ml}$  AML and ATOR in the buffer solution of pH 6 at different potential scan rates from 40 to 400 mV/s. Figs. 5b and c reveal the linear relationship between  $\log I_{pa}$  and  $\log v$  indicating a mixed adsorption-diffusion controlled process on the surface of the modified electrode. The equation for AML is

$$\text{AML: } \log I_{pa} = 1.034 \log v - 1.449 \quad (R^2 = 0.983)$$

and the equation for ATOR is

$$\text{ATOR: } \log I_{pa} = 0.974 \log v - 1.132 \quad (R^2 = 0.981)$$



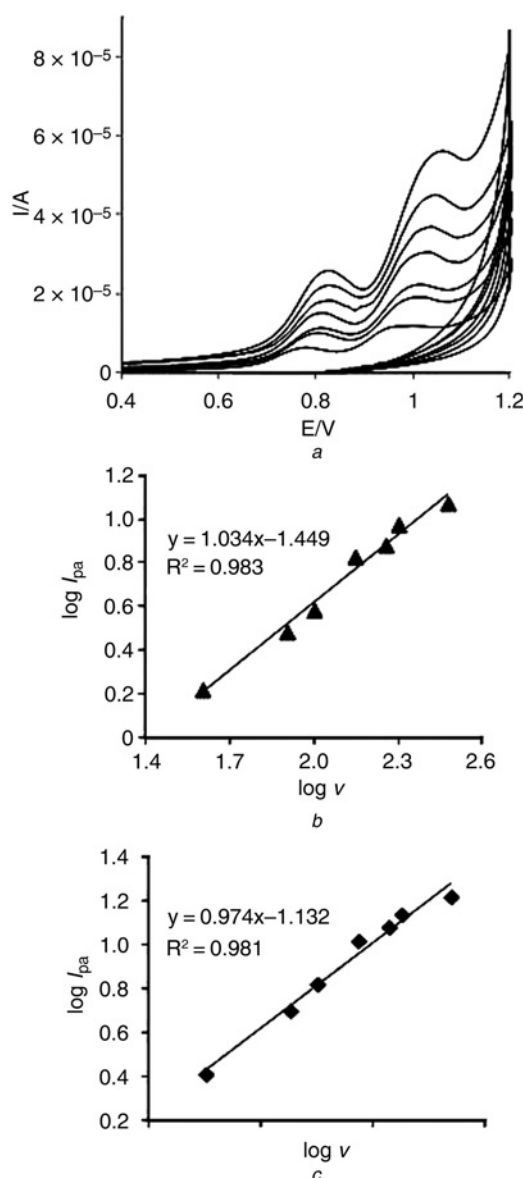
**Figure 4** DPVs of 20  $\mu\text{g/ml}$  of AML and ATOR at MWCNTs:G paste electrode in various pHs from 3 to 7: (a) 3, (b) 4, (c) 5, (d) 6, (e) 7 (Fig. 4a), and dependence of oxidation peak potential ( $E_{pa}$ ) with pH solution for (a) AML and (b) ATOR (Fig. 4b)

**3.3. Method validation:** To develop a method for determination of the drugs, we selected differential pulse voltammetry since the peaks are sharper and better than those obtained by cyclic voltammetry. The variation of oxidation peak current with different concentrations of AML and ATOR (1:1) was studied simultaneously using the MWCNTs:G paste electrode in buffer solution (pH 6). The voltammograms are shown in Fig. 6. A linear range of 2.5–100  $\mu\text{g/ml}$  was obtained for AML and ATOR in Fig. 6b and c with the relative standard deviation (RSD) values for three curves ranging from 2.7–7.1% for AML and 1.8–8.3% for ATOR. The linear regression equation for AML is

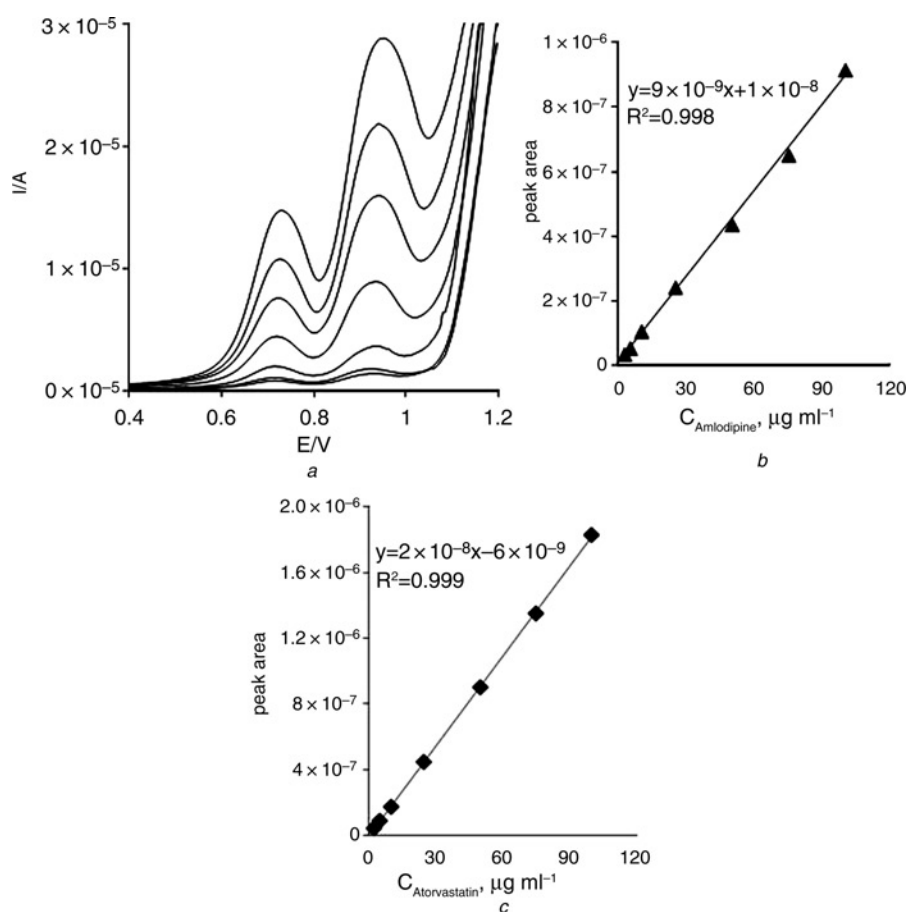
$$\text{AML: } I_{pa} = 9 \times 10^{-9} C + 1 \times 10^{-8} \quad (R^2 = 0.998)$$

and for ATOR it is

$$\text{ATOR: } I_{pa} = 2 \times 10^{-8} C - 6 \times 10^{-9} \quad (R^2 = 0.999)$$



**Figure 5** CVs of 20  $\mu\text{g/ml}$  of AML and ATOR at MWCNTs:G paste electrode in buffer solution (pH 6) at various scan rates from inner to outer: 40, 80, 100, 140, 180, 200, 300 mV/s (Fig. 5a), and plots of  $\log I_{pa}$  against  $\log v$  for AML (Fig. 5b) and ATOR (Fig. 5c)



**Figure 6** DPVs of AML and ATOR at MWCNTs:G paste electrode in different concentrations from inner to outer: 2.5, 5, 10, 25, 50, 75, 100 µg/ml (Fig. 6a), and dependence of oxidation peak area with drug concentration for AML (Fig. 6b) and ATOR (Fig. 6c)

The quantitation and detection limits for AML and ATOR are 2.5 and 1 µg/ml, respectively. Repeatability (intra-day) of the method was examined with each of the three differential pulse voltammograms (DPVs) of the three samples containing

different concentrations in the linear range. Reproducibility (inter-day) was investigated by electrochemical determination of the three samples with different concentrations in the linear range. The results are shown in Tables 1 and 2.

**Table 1** Repeatability (intra-day) of method with samples containing different concentrations in linear range

AML concentration, µg/ml	Intra-day ( <i>n</i> = 3)		Inter-day ( <i>n</i> = 3)	
	<i>I</i> <sub>pa</sub> , A	RSD, %	<i>I</i> <sub>pa</sub> , A	RSD, %
5	$5.37 \times 10^{-8}$	4.7	$5.48 \times 10^{-8}$	2.6
25	$2.42 \times 10^{-7}$	7.1	$2.60 \times 10^{-7}$	6.2
75	$6.52 \times 10^{-7}$	3.36	$6.80 \times 10^{-7}$	2.22

**Table 2** Reproducibility (inter-day) of method with samples containing different concentrations in linear range

ATOR concentration, µg/ml	Intra-day ( <i>n</i> = 3)		Inter-day ( <i>n</i> = 3)	
	<i>I</i> <sub>pa</sub> , A	RSD, %	<i>I</i> <sub>pa</sub> , A	RSD, %
5	$8.87 \times 10^{-8}$	6.9	$8.55 \times 10^{-8}$	1.12
25	$4.50 \times 10^{-7}$	1.8	$4.79 \times 10^{-7}$	7.5
75	$1.35 \times 10^{-6}$	2.5	$1.33 \times 10^{-6}$	2.75

**Table 3** Different electrodes used for determination of AML and ATOR

Drug	Electrode	Medium/pH	Limit of detection	Applications	Reference
amlodipine besylate	CNT/pyrolytic graphite	buffer/7.2	$5.0 \times 10^{-9}$ M	pharmaceutical/human urine	[11]
amlodipine besylate	carbon paste	buffer/11	$2 \times 10^{-10}$ M	pharmaceutical	[12]
amlodipine besylate	GC	buffer/11	$1.40 \times 10^{-8}$ M	pharmaceutical, human urine/serum	[13]
atorvastatin calcium	hanging mercury drop	—	0.037 µg/ml	pharmaceutical, plasma	[17]
atorvastatin calcium	boron-doped diamond	0.1 M H <sub>2</sub> SO <sub>4</sub>	$2.27 \times 10^{-7}$ M	pharmaceutical, human urine/serum	[18]
atorvastatin calcium	GC	0.1 M H <sub>2</sub> SO <sub>4</sub>	$2.11 \times 10^{-7}$ M	pharmaceutical, human urine/serum	[18]
amlodipine besylate	GC	buffer/5	$8.53 \times 10^{-7}$ M	pharmaceutical	[19]
atorvastatin calcium	GC	buffer/5	$4.70 \times 10^{-7}$ M	pharmaceutical	[19]
AML/ATOR	MWCNTs:G	buffer/6	1 µg/ml	tablet	this work



3.4. Assay of AML and ATOR in tablets: The proposed method was applied for the determination of AML and ATOR in a generic available tablet. The results of the assays ( $n=3$ ) undertaken yielded 98.8% (%RSD = 5.7%) and 101.5% (%RSD = 5.44%) of the label claim for AML and ATOR, respectively. These results indicated that the method is selective for the analysis of both AML and ATOR without interference from the excipients used to formulate and produce these tablets. The most important reported electrochemical methods for determination of AML and ATOR are summarised in Table 3.

**4. Conclusion:** In this work, the response of the MWCNTs/G paste electrode to electrochemical oxidation of AML and ATOR was investigated. The electrode was successfully used for determination of AML and ATOR in tablets directly, without any separation steps. The results of the study in this procedure are sensitive and selective using the MWCNTs/G paste electrode. Sample preparation is easy and the method is reproducible.

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