Novel Validated Chromatographic Method for Determination of Some Anti-hypertensive Drugs

M. Farouk[1], O. Abd ELAziz[1], A. Hemdan[*2], M. Shehata[2]

[1] Analytical Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.
[2] Pharmaceutical Chemistry Department,. Faculty of Pharmacy, Ahram Canadian University, 6th October, Egypt.
[*]hemmdan@yahoo.com

Abstract: Accurate, precise and reproducible isocratic RP-HPLC method was developed and subsequent validated for the analysis of Torasemide (I), Irbesartan (II) and Olmesartan medoxomil (III) at ambient temperature, using Atlantis 4.6 mm x 250 mm RP-C18 Column, with a flow rate of 1.5 ml.min^{-1}, and UV. detector at 288 nm and 260 nm for (I) and (II and III), respectively. By adopting the mentioned chromatographic technique, (I) and (III) were determined in the presence of their acidic and alkaline-degradates separately as stability-indicating methods utilizing phosphate buffer pH = 3:acetonitrile (60:40, v/v), phosphate buffer pH = 3.2:acetonitrile (60:40, v/v) as a mobile phase, respectively, while (II) was determined in presence of Hydrochlorothiazide (HCTZ), using phosphate buffer pH = 4:acetonitrile (70 :30, v/v). All the proposed methods were validated according to the International Conference on Harmonization (ICH) guidelines and successfully applied to determine the mentioned studied drugs in pure form, in laboratory prepared mixtures and in pharmaceutical preparations. The obtained results were statistically compared to the reference methods of analysis [for I and "II and III", respectively] and no significant differences were found.
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Keywords: Torasemide, Irbesartan, Olmesartan medoxomil, High Performance Liquid Chromatography, Stability Indicating method

1. Introduction:

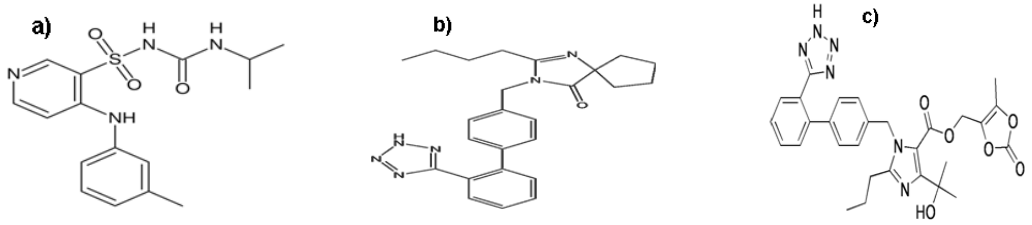


Figure (1): Chemical structure of: a) Torasemide, b) Irbesartan, c) Olmesartan medoxomil

Torasemide (I) is (1-isopropyl-3-[[4-(3-methylphenylamine) pyridine]-3-sulfonyl] urea) a loop diuretic, mainly used at low doses for the management of hypertension, where in large doses used for management of oedema associated with congestive heart failure[(1)]. Irbesartan (II) is 2-butyl-3-[[2-(tetrazol-5-yl) biphenyl-4-yl]-methyl]-1,3-diazaspiro[4.4]non-1-en-4-one, acts as an angiotensin-II receptor antagonist, used mainly for the treatment of hypertension[(2)], while, Olmesartan medoxomil (III) is 5-methyl-2-oxo-1,3-dioxolen-4yl) methyl-4-(1-hydrxy-1-methylethyl)-2-propyl-1-[4-(2-(tetrazole-5yl)phenyl] methylimidazole 5 carboxylate, used for the treatment of hypertension by the same mechanism as (II)[(3)]. The ICH-guidelines[(4)] recommends performing stress-testing of the drug substance that can help in identifying the likely degradation-products, also can be useful in establishing the degradation-pathways and validating the stability-indicating power of the analytical procedures used[(5)]. Stability-indicating methods can be used for evaluating the drug in the presence of its-degradation products, excipients and additives [(6)]. Several methods have been reported for the determination of (I), including colorimetry[(7)], differential-pulse adsorptive stripping voltammetry[(8)], capillary zone electrophoresis (CZE)[(9,10)], gas chromatography[(11)], micellar liquid chromatography[(12)], and high-performance liquid chromatography[(13-22)]. Alone or in combination with HCTZ, Irbesartan has been determined by derivative spectrophotometry[(23-27)], kinetic Spectrophotometry[(28)], spectrofluorimetry[(29)], colorimetry[(30)], adsorptive stripping voltammetric[(31)],

A differential pulse (DP) and square wave (SW) voltammetry[32], capillary zone electrophoresis[33-35], micellar electrokinetic chromatography[36], and high-performance liquid chromatography[37-43]. While for Olmesartan medoxomil (III), several methods have been reported for its determination, either alone or in combination with HCTZ, these methods were based on absorption ratio spectrophotometry[44], ratio spectra derivative and zero-crossing difference spectrophotometry[45,46], derivative spectrophotometry[47], direct spectrophotometry[48,49], capillary zone electrophoresis[50], high performance thin layer chromatographic method[51,52], and high-performance liquid chromatography[52-59].

The main goal of this work is to establish accurate, precise, rapid and reproducible isocratic chromatographic methods for determination '(I), and (III) in presence of their-degradates and simultaneous determination of (II) in binary mixture with HCTZ that can be adopted as a technique for the routine quality control analysis of these drugs in raw material and pharmaceutical preparations as well as for stability studies.

2. Experimental:

2.1. Chemicals and reagents

Torasemide was kindly provided by Apex Pharma-Egypt and certified to contain 99.70%. Examide® tablets: batch number: MT1120410, manufactured by Apex Pharma-Egypt Company. Each tablet was labeled to contain 20 mg of Torasemide. Irbesartan was kindly obtained by Sanofi-Aventis Egypt and certified to contain 99.90%. Co-Approval® tablets: batch number: 1145, manufactured by Sanofi-Aventis Egypt. Each tablet was labeled to contain 300 mg of Irbesartan and 12.5 mg Hydrochlorothiazide. Hydrochlorothiazide (HCTZ) was kindly provided by Multi-Pharma Egypt and certified to contain 99.50%. Olmesartan medoxomil was kindly provided by Apex Pharma-Egypt and certified to contain 99.70%. Erastapex® tablets: batch number: MT3241009, manufactured by Apex Pharma-Egypt Company. Each tablet was labeled to contain 40 mg of Olmesartan medoxomil.

Acetonitrile, ethyl acetate, methanol and bi-distilled water (Riedel-dehaen, Sigma-Aldrich, Germany), hydrochloric acid, sodium hydroxide and sulfuric acid (BDH), each 'aqueous 0.1, '0.1 and 6.6' and 5M. 'Monobasic potassium phosphate and O-phosphoric acid (Adwic)' and triethylamine (Fluka).

All chemical and reagents used through this work are of chromatographic analytical grade. Bi-distilled water is used throughout the whole work and is indicated by the word "water".

2.2. Instruments

The HPLC Schimadzu LC-Lab Solution instrument comprised an isocratic pump model Schimadzu LC–20AD, connected to PC and software (LC-Solution), SIL 20A auto-sampler - Schimadzu injector and a Schimadzu SPD20A UV detector. The chromatographic separation was performed using Atlantis C18 column (5µm, 250 x 4.6 mm i.d.) at ambient temperature.

Ultrasonic vibrator, Crest Ultrasonic-Tru / Sweep; Model 575TAE, N. Y, U.S.A.

A (Jenway 3510, UK) pH-meter, equipped with combined glass electrode for pH adjustment.

2. 3. Standard Solutions

2.3.1. Standard solutions of the investigated drugs

Stock standard solutions of (I, II and III), each having concentration of (0.5 $mg.ml^{-1}$), were prepared in methanol, respectively, where all the prepared solutions were used as a working standard solutions.

2.3.2. Standard solution of Hydrochlorothiazide

Stock standard solution of HTCZ having concentration of (0.5 $mg.ml^{-1}$), was prepared in methanol, where it was used as a working standard solution.

2.3.3. Standard solution of degradates

2.3.3.1. Standard solution of Torasemide degradates

Standard stock solution of acid-degradate, was prepared, by mixing 50.0 mg of (I) with 20 ml of 5.0 M sulfuric acid, refluxing for 12 hours, cooling, then neutralizing the media with 6.6 M NaOH, and making the volumes to 100 ml with methanol to obtain a concentration of 500 $\mu g.ml^{-1}$.

2.3.3.2. Standard solution of Olmesartan degradates

Standard stock solution of alkaline-degradate, was prepared, by mixing 50.0 mg of (III) with 10 ml of 0.1M NaOH, refluxing for 20 ninutes, cooling, then neutralizing the media with 0.1M HCl and making the volumes to 100 ml with methanol to obtain a concentration of 500 $\mu g.ml^{-1}$.

Complete degradation was checked by TLC using silica gel 60 F254 plates and chloroform: ethyl acetate: methanol [8 : 8 : 4] as a mobile phase.

2.4. Procedures:

Stationary phase, Atlantis C18 column (5µm, 250 x 4.6 mm i.d.), acetonitrile: phosphate buffer 'pH 3' in a ratio (40:60, v/v), acetonitrile: phosphate buffer 'pH 4' in a ratio (30:70, v/v) and acetonitrile: phosphate buffer 'pH 3.2' in a ratio (40:60, v/v) as 'degassed and filtered' mobile phases with a flow rate of 1.5 $ml.min^{-1}$ were the chromatographic conditions

adopted for determination of Torasemide, and 'Irbesartan and Olmesartan medoxomil' using UV detection at 288 and 260 nm, respectively. Construction the calibration curves were performed by transferring aliquots of each working standard solution separately into a series of 25 ml volumetric flasks and diluting with the mobile phase to the volume, having a concentration range of 0.2 – 25, 0.1-20, 0.5-30 µg ml^{-1} for the investigated drugs, respectively. Under the previously mentioned chromatographic conditions, 100µl-volume from each solution was injected in triplicate, using HCTZ and Torasemide as an internal standards in a concentration of 50 and 4 µg ml^{-1} for determination of I and (II and III), respectively. The obtained average peak area for each concentration of each drug was plotted versus concentration and the regression equation was then computed.

2.5. Assay of the pharmaceutical formulations:

Five tablets of Examide®, Co-Approval® and Erastapex® were accurately weighed and finely powdered separately. Portion of each powder equivalent to 10 mg (I, II and II) were accurately weighed, transferred to 100 ml volumetric flask, shaked for 15-minutes with 50 ml methanol, filtered completed to the volume with methanol, to obtain a concentration of 100 µg.ml^{-1} and then the mentioned procedure under 2.4. was adopted.

3. Results and Discussion:

3.1. Method development:

Torasemide and Olmesartan

Separation of I and III from their degradation-products has been performed on Atlantis C18 column (5µm, 250 x 4.6 mm i.d.). The proportion of the mobile phase components was optimized to reduce each of 'retention time and tailing' and to enable good resolution from its-degradates. At high acetonitrile ratio, retention time of different components decrease but with excessive tailing of its peak. High resolution was obtained by using acetonitrile: phosphate buffer 'pH 3' in a ratio (40:60, v/v) and acetonitrile: phosphate buffer 'pH 3.2' in a ratio (40:60, v/v) as a mobile phase, with a flow rate 1.5 $ml.min^{-1}$, and detection at 288 and 260 nm, respectively, where the maximum sensitivity was observed. The average retention time was 3.56 ± 0.03 and 3.85 ± 0.03 min, respectively, as shown in (Figures 2, 3, 6 and 7).

Irbesartan

Separation of ibesartan from HCTZ in binary-mixture has been performed on Atlantis C18 column (5µm, 250 x 4.6 mm i.d.). The proportion of the mobile phase components was optimized to reduce each of 'retention time and tailing' and to enable good resolution of II from HCTZ. At high acetonitrile ratio, retention time of different components decrease but with excessive tailing of its peak. High resolution was obtained by using acetonitrile: phosphate buffer 'pH 4' in a ratio (30:70, v/v) as a mobile phase, with a flow rate 1.5 $ml.min^{-1}$ and detection at 260 nm, where the maximum sensitivity was observed. The average retention time was 9.89 ± 0.03 min as shown in (Figures 4-5).

3.2. Methods validation.

ICH-guidelines[4)] for method validation were followed. All validation parameters are shown in (Table 1).

3.2.1. Linearity:

A linear correlation was obtained between peak area and concentration of (I, II and III) in a range of 0.2 – 25, 0.1-20, 0.5-30 µg mL^{-1} with correlation coefficient [r] = 0.9998, 0.9999 and 0.9999, respectively.

3.2.2. Accuracy:

Accuracy of the proposed methods was tested by analyzing freshly prepared solutions of the studied drugs in triplicate. The recovery percent and standard deviations (S.D.) revealed excellent accuracy. The results obtained by applying the proposed chromatographic methods were statistically compared with those results obtained by the reference methods[(60-62)]. It was concluded that with 95% confidence, there is no significant difference between them since the calculated *t* and *F* values are less than the theoretical values[(63)] (Tables 2a-2c).

3.2.3. Repeatability and reproducibility:

The intra- and inter-day precision was evaluated by assaying freshly prepared solutions in triplicate, as shown in (Table 1).

3.2.4. Specificity:

The specificity of the adopted HPLC method was illustrated by the complete separation of the studied drugs, as shown in (Figures 2-7). The Rs-values from main (acid alkaline-degradates) and from HCTZ were always above 2, which ensured complete separation. Furthermore, I and III were determined in solutions of laboratory prepared mixtures containing their acid and alkaline-degradates and III from HCTZ by the proposed method. The Recovery % and R.S.D. % proved the high specificity of these methods (Table 3).

3.2.5. Robustness and system suitability of the HPLC method:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected after slight but deliberate changes in the analytical conditions. Separation of the studied drugs from their different-degradates or from other drug in-combination was performed under these conditions. There was slight decrease or increase in the Rs-values of all peaks. However, the calculated Rs-values were always above 2, ensuring complete separation. The system suitability parameters of HPLC method were evaluated[64] (Tables 4a-4c).

3.3. Standard addition technique:

The proposed methods were applied for the determination of the studied drugs in the commercial tablets. The results shown in (Tables 5a-5c), were satisfactory and with good agreement with the labeled amount. Moreover, to check the validity of the adopted proposed methods, the standard addition method was applied by adding known amounts of the studied drugs to the previously analyzed tablets. The recoveries were calculated by comparing the concentration obtained from the spiked samples with that of the pure drug. The results of the commercial tablets analysis and the standard addition method (recovery study) (Tables 5a-5c) suggested that there is no interference from any excipients, which are normally present in tablets.

3.4. Identification of Torasemide acid-degradate and Olmesartan medoxomil alkaline-degradate:

3.4.1. Identification of Torasemide acid-degradate

Structure elucidation of Torasemide acid-degradate exhibiting terminal amide bond cleavage, resulting in formation of hydroxyl and carbonyl groups, which was explained by utilizing FT-IR "Fourier transform spectroscopy" and M.S., techniques. In the FT-IR technique, the acid-degradate showed a similar absorption pattern to (I) except the appearance of the acid-degradate bands at 3463.4 and 1735.7 cm^{-1}, respectively, while in M.S., two peaks were delivered at m/z 59 and 307, respectively, (figures 8a-8c).

3.4.2. Identification of Olmesartan alkaline-degradate

By the same manner, the structure elucidation of Olmesartan alkaline-degradate exhibiting ester bond cleavage, resulting in formation of hydroxyl and carbonyl groups, which was explained by utilizing FT-IR "Fourier transform spectroscopy" and M.S., techniques. In the FT-IR technique, the alkaline-degradate showed a similar absorption pattern to (III) except the disappearance of the ester carbonyl band at 1737.2 cm^{-1} and the appearance of the corresponding Hydroxyl and carbonyl bands of the carboxylic group of the degradation product at 3423.5 and 1712.7 cm^{-1}, respectively, on the other hand, mass spectrum of the alkaline degradation product exhibited two new peaks at m/z 130 and 446, respectively, (figures 9a-9c).

4- Conclusion:

The proposed HPLC methods were precise, specific, accurate and reproducible , where Torasemide, Irbesartan and Olmesartan can be determined in bulk powder and in pharmaceutical formulations without interference from excipients present, as well as in the presence of their different-degradates or other drug in-combination by the d. ICH-guidelines were followed throughout method validation and the suggested methods can be applied for routine quality control analysis and stability studies.

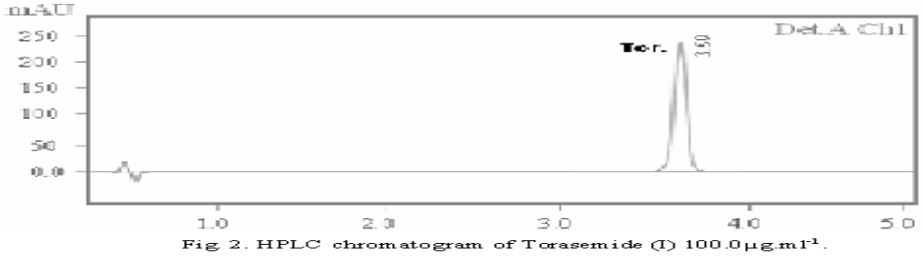


Fig 2. HPLC chromatogram of Torasemide (I) 100.0 µg.ml^{-1}.

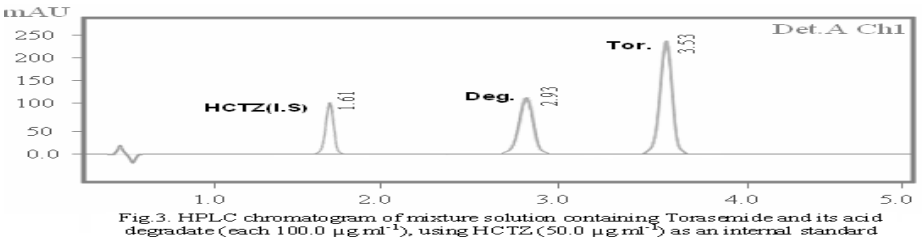


Fig.3. HPLC chromatogram of mixture solution containing Torasemide and its acid degradate (each 100.0 µg.ml^{-1}), using HCTZ (50.0 µg.ml^{-1}) as an internal standard

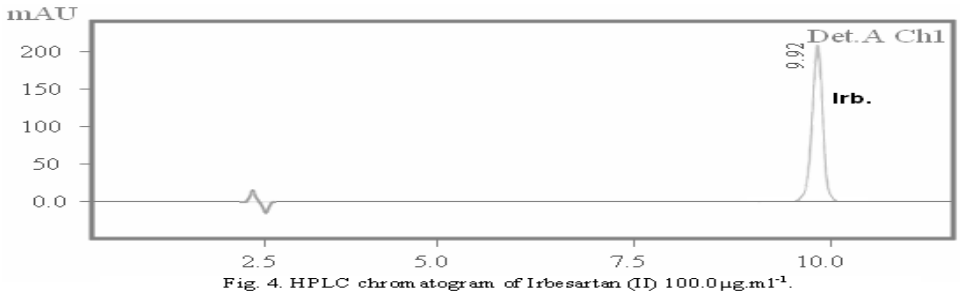


Fig. 4. HPLC chromatogram of Irbesartan (II) 100.0 µg.ml^{-1}.

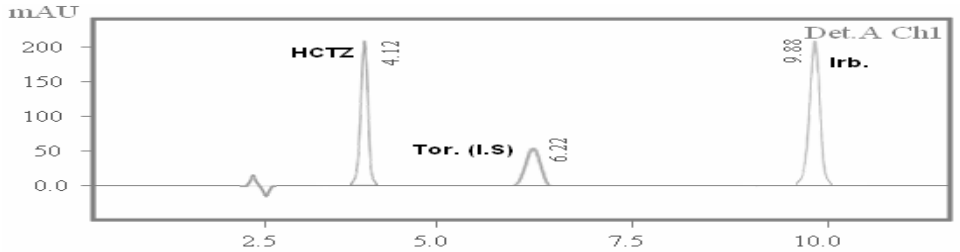


Fig.5. HPLC chromatogram of mixture solution of Irbesartan (II) in presence of Hydrochlorothiazide (each 100.00 µg.ml^{-1}), using Torasemide (4.00 µg.ml^{-1}) as an internal standard.

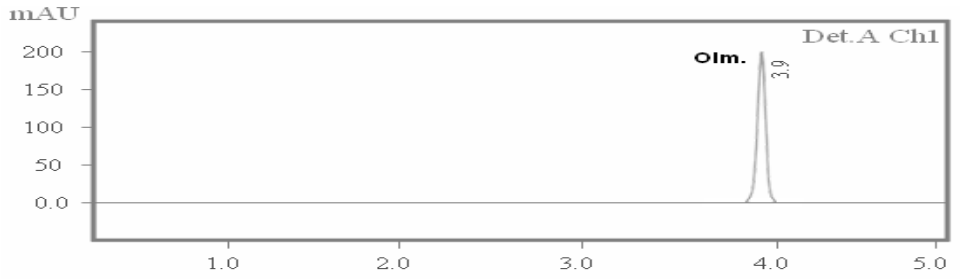


Fig. 6. HPLC chromatogram of Olmesartan (III) 50.0 µg.ml^{-1}.

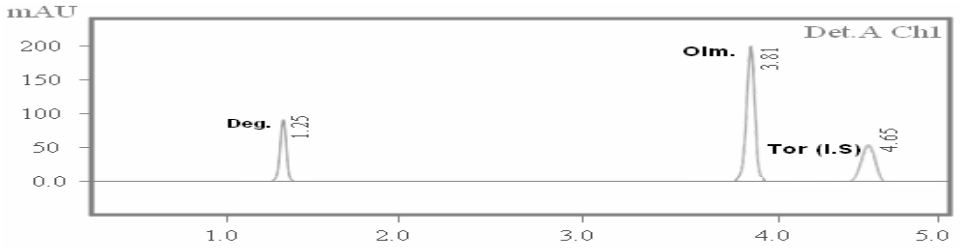


Fig. 7. HPLC chromatogram of mixture solution containing Olmesartan (III) and its alkaline degradate (each 50.0 µg.ml^{-1}), using Torasemide (4.0 µg.ml^{-1}), as an internal standard.

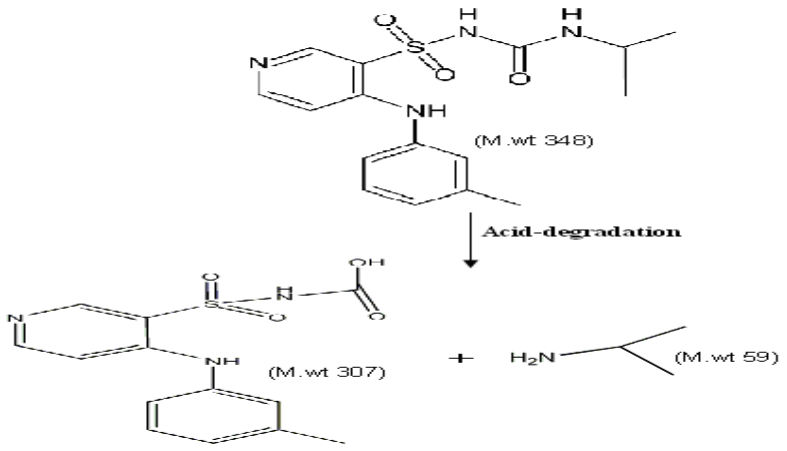
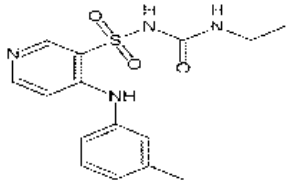


Fig. 8.a. Suggested degradation pathway of Torasemide

Torasemide (intact):



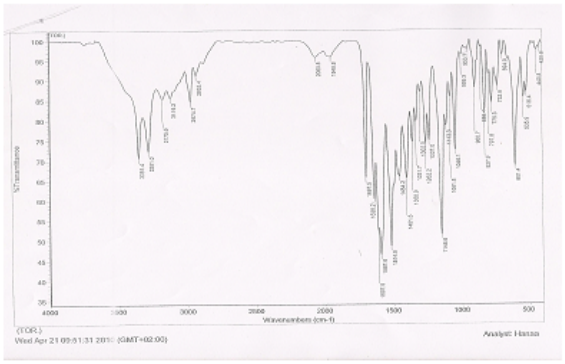


Figure 8.b.1: IR spectrum of the Intact Torasemide

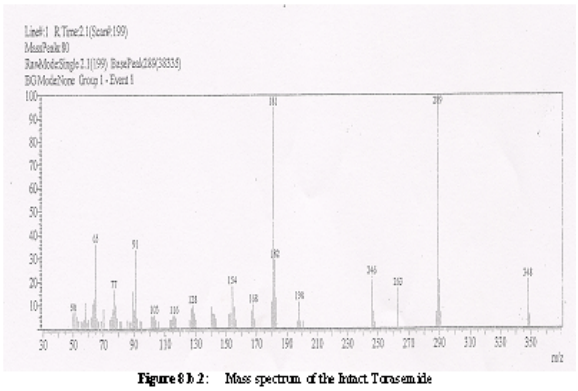
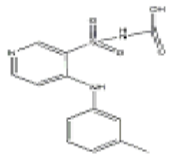
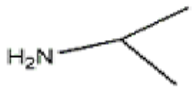


Figure 8.b.2: Mass spectrum of the Intact Torasemide

Torasemide (acid-degradate):





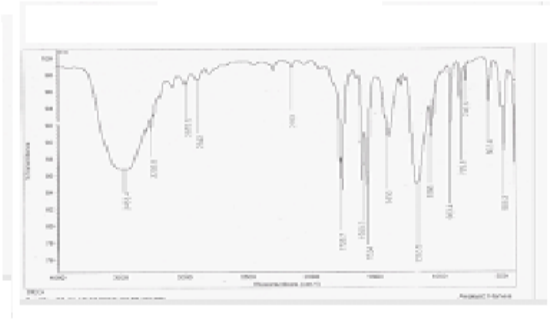


Figure 8.c.1.: IR spectrum of the acid degradate of Torasemide

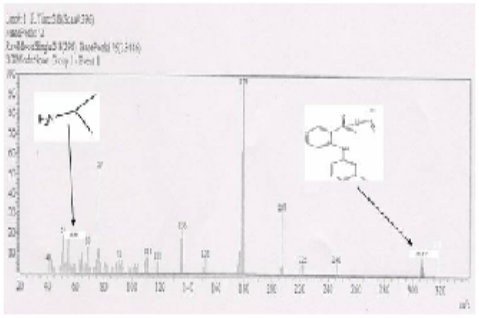


Figure 8.c.2.: Mass spectrum of the acid degradate of Torasemide

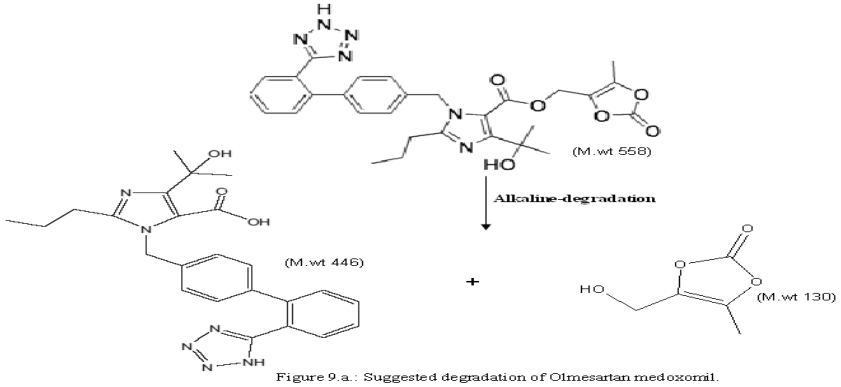
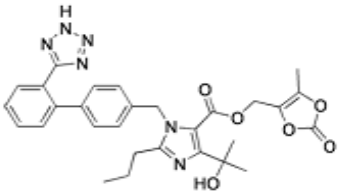


Figure 9.a.: Suggested degradation of Olmesartan medoxomil.

Olmesartan (intact):



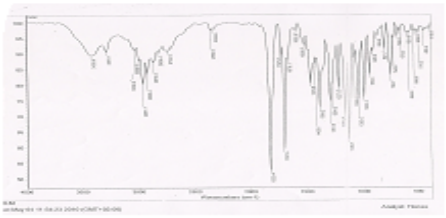


Figure 9.b.1. IR spectrum of Olmesartan

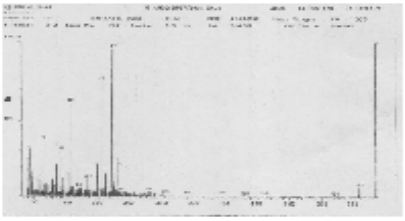
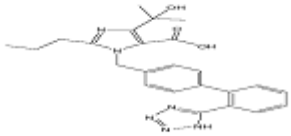
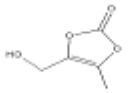


Figure 9.b.1. Mass spectrum of Olmesartan

Olmesartan (alkaline-degradate):





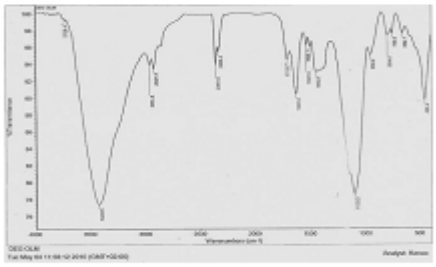


Figure 9.c.1 IR spectrum of the Olmesartan alkaline –degradate.

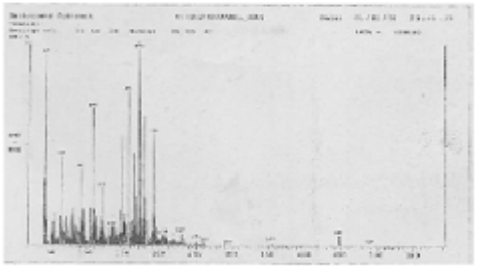


Figure 9.c.2 Mass spectrum of the Olmesartan alkaline –degradate.

Table 1: Validation report of the proposed HPLC methods for determination of Torasemide (I), Irbesartan (II) and Olmesartan (III).

Parameters	Torasemide	Irbesartan	Olmesartan
Linearity	0.2-25μg.ml^{-1}	0.1-20μg.ml^{-1}	0.5-30μg.ml^{-1}
Intercept	0.0115	0.053	0.0485
Slope(b)[a]	0.0381	0.7189	0.6103
Correlation coefficient (r)	0.9998	0.9999	0.9999
Accuracy[b]	99.97±0.97	100.59±0.74	100.71±0.57
Precision:			
Repeatability[b]	100.40 ± 0.430	100.50 ± 0.670	99.80 ± 0.480
Intermediate precision[b]	100.70 ± 0.610	99.37 ± 0.750	100.80 ± 0.610

[a]Regression equation = "A = a + bc" for HPLC; where "A" = peak area and "c" = the concentration (μg.ml^{-1}).
[b]Mean ± S.D.

Table 2a: Statistical comparison between the proposed method and the reference method[60] for the determination of Torasemide (I).

Methods	Parameters					
	Mean	S.D.	n	Variance	Student's *t*-test	F test
Reference method	100.2	0.48	6	0.230	-	-
HPLC	99.97	0.97	6	0.940	0.52 (2.23)	4.08 (5.19)

Values in parenthesis are the theoretical values of *t* and F at P=0.05.

Table 2b: Statistical comparison between the proposed methods and the reference method [61] for the determination of Irbesartan(II).

Methods	Parameters					
	Mean	S.D.	n	Variance	Student's *t*-test	F test
Reference method	99.8	0.65	6	0.422	-	-
HPLC	100.59	0.74	6	0.547	1.96 (2.23)	1.29 (5.19)

Values in parenthesis are the theoretical values of *t* and F at P=0.05.

Table 2c: Statistical comparison between the proposed methods and the reference method[62] for the determination of Olmesartan medoxomil (III).

Methods	Parameters					
	Mean	S.D.	n	Variance	Student's *t*-test	F test
Reference method	100.5	0.47	6	0.220	-	-
HPLC	100.71	0.57	6	0.324	0.69 (2.23)	1.47 (5.19)

Values in parenthesis are the theoretical values of *t* and F at P=0.05.

Table 3: Determination of Torasemide (I), Irbesartan (II) and Olmesartan (III) in laboratory prepared mixtures containing their degradates by the proposed HPLC methods:

Sample no.	% Degradates	% Recovery*		
		Torasemide	Irbesartan	Olmesartan
1	20	99.34	101.5	101.61
2	40	101.97	100.66	100.96
3	60	99.08	100.8	101.29
4	80	101.71	99.27	100.63
5	100	99.29	99.36	100.14
6	120	100.92	101.22	100.79
Mean		100.38	100.47	100.9
R.S.D.%		1.30	0.938	0.51

*Mean of three determinations.

Table 4a: Results from robustness testing of the proposed HPLC method for Torasemide (I).

Conditions	**R_t**	**N**	**T**	**R_S**
Flow rate:				
1.3 ml.min^{-1}	4.25	1470	1.21	2.4
1.5 ml.min^{-1}	3.53	3073	1	2.6
Mobile phase composition:				
phosphate buffer :acetonitrile (60:40, v/v)	3.53	3073	1	2.6
phosphate buffer :acetonitrile (70:30, v/v)	5.21	2450	1.12	2.3
pH:				
3	3.53	3073	1	2.6
3.5	4.68	1680	1.1	2.3

Table 4b: Results from robustness testing of the proposed HPLC method for Irbesartan

Conditions	R_t	N	T	R_S
Flow rate:				
1.3 $ml.min^{-1}$	12.6	10354	1.2	14.3
1.5 $ml.min^{-1}$	9.88	12284	1	16.2
Mobile phase composition:				
phosphate buffer :acetonitrile (60:40, v/v)	8.12	11542	1.4	15.4
phosphate buffer :acetonitrile (70:30, v/v)	9.88	12284	1	16.2
pH:				
3.5	8.23	10563	1.25	13.2
4	9.88	12284	1	16.2

Table 4c: Results from robustness testing of the proposed HPLC method for Olmesartan

Conditions	R_t	N	T	R_S
Flow rate:				
1.3 $ml.min^{-1}$	5.21	10268	1.24	13.9
1.5 $ml.min^{-1}$	3.81	12588	1	15
Mobile phase composition:				
phosphate buffer :acetonitrile (60:40, v/v)	3.81	12588	1	15
phosphate buffer :acetonitrile (70:30, v/v)	4.8	11563	1.21	14.1
pH:				
2.8	3.5	11563	1.1	14.4
3.2	3.81	12588	1	15

Table 5a: Determination of Torasemide (I) in pharmaceutical formulation using the proposed HPLC and application of standard addition technique:

Pharmaceutical Preparation	Found*	Standard Addition Technique			
	%± S.D	Taken µg ml^{-1}	Taken µg ml^{-1}	Taken µg ml^{-1}	Taken µg ml^{-1}
Examide® 20 mg Batch No: MT1120410	100.60 ± 0.36	10	2	2.006	100.3
			5	5.06	101.2
			7	7.035	100.5
			10	9.98	99.8
			12	12.024	100.2
			15	15.045	100.3
Mean ± S.D					100.38 ± 0.46

* The mean percentage recovery of 4-separate determinations for the pharmaceutical preparation

Table 5b: Determination of Irbesartan in pharmaceutical formulation using the proposed HPLC and application of standard addition technique:

Pharmaceutical Preparation	Found*	Standard Addition Technique			
	%± S.D	Taken µg ml^{-1}	Taken µg ml^{-1}	Taken µg ml^{-1}	Taken µg ml^{-1}
Co-Approvel® 300mg/12.5mg Batch No: 1145	101.5 ± 0.56	10	1	1.002	100.2
			2	2.002	100.1
			4	3.992	99.8
			6	6.018	100.3
			8	8.096	101.2
			10	9.98	99.8
Mean ± S.D					100.23±0.52

* The mean percentage recovery of 4-separate determinations for the pharmaceutical preparation

Table 5c: Determination of Olmesartan (III) in pharmaceutical formulation using the proposed HPLC and application of standard addition technique:

Pharmaceutical Preparation	Found*	Standard Addition Technique			
	%± S.D	Taken µg ml^{-1}	Taken µg ml^{-1}	Taken µg ml^{-1}	Taken µg ml^{-1}
ERASTAPEX® 40mg Batch No: MT3241009	100.7 ± 0.84	10	1	1.003	100.3
			2	1.99	99.5
			4	3.984	99.6
			6	6.048	100.8
			8	8.04	100.5
			10	9.96	99.6
Mean ± S.D					100.05±0.55

* The mean percentage recovery of 4-separate determinations for the pharmaceutical preparation

Corresponding author
A. Hemdan
Pharmaceutical Chemistry Department,. Faculty of Pharmacy, Ahram Canadian University, 6th October, Egypt.
hemmdan@yahoo.com

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9/1/2010