Simple Novel Spectrophotometric and Spectrofluorimetric Methods for Determination of Some Anti-hypertensive Drugs

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Abstract: Accurate, precise and selective spectrophotometric and spectrofluorimetric methods were developed and subsequently validated for determination of Torasemide (I), Irbesartan (II) and Olmesartan medoxomil (III), where (I) could be determined in presence of its acidic-degradate as stability indicating method, utilizing derivative ratio spectrophotometry, also in human plasma it could be determined by spectrofluorimetric method, (II) could be determined in a binary mixture with Hydrochlorothiazide (HCTZ) by simultaneous determination, utilizing ratio subtraction and spectrofluorimetric techniques, while (III) could be determined in presence of its alkaline-degradate as stability indicating method, utilizing derivative ratio and pH-induced difference spectrophotometric technique, also in a binary mixture with Hydrochlorothiazide (HCTZ), it could be determined by simultaneous determination, using ratio subtraction and spectrofluorimetric methods. All the proposed novel methods were validated according to International Conference of Harmonization (ICH) guide lines 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, II and III, respectively] and no significant difference were found.

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1. Introduction:

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

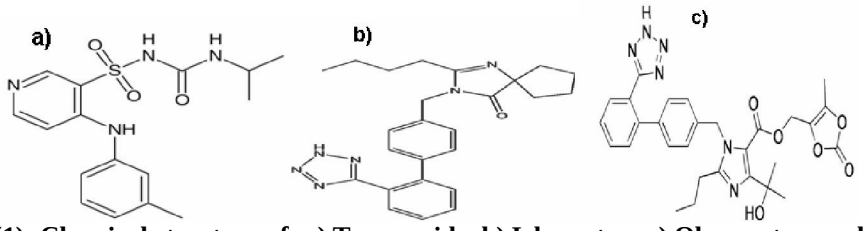

Figure (1): Chemical structure of: a) Torasemide, b) Irbesartan, c) Olmesartan medoxomil

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-guide lines[(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 spectrophotometric and spectrofluorimetric methods for determination of (I) and (III) in presence of their-degradates, also simultaneous determination of (II) and (III) separately in binary mixture with HCTZ, which can be adopted for the routine quality control analysis of the investigated drugs in raw material, and pharmaceutical preparations as well as for stability studies.

In this paper, between the adopted new spectrophotometric methods, we utilized a ratio subtraction spectrophotometric technique for simultaneous determination of two binary mixtures [(II) and (III)] each with HCTZ.

This technique has the following theory:

A mixture of two drugs X and Y with overlapping spectra can be resolved by ratio subtraction, if the spectrum of one drug, say (Y) is extended more than the other, say (X) can be determined by dividing the spectrum of the mixture by a certain concentration of Y as a divisor (Y'). The division will give a new curve that is represented by:

$$X / Y' + \text{Constant}$$

If the constant is subtracted, then the new curve obtained is multiplied by Y', the original curve of X is obtained.

This can be summarized in the following equations:

$$(X+Y) / Y' = (X / Y') + (Y / Y') = X / Y' + \text{Constant}$$

$$X / Y' + \text{Constant} - \text{Constant} = X / Y'$$

$$X / Y' \times Y' = X$$

The constant can be determined directly from the curve (X+Y) / Y' by the straight line that is parallel to the wavelength axis in the region where Y is extended.

2. Materials and methods

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. Erastapex Plus® batch number MT0280110, manufactured by Apex Pharma-Egypt Company. Each tablet was labeled to contain 40 mg Olmesartan and 12.5 mg HCTZ.

Boric acid (Adwic), Bi-distilled water, Chloroform, Ethyl acetate and Methanol (Riedel-dehaen, Sigma-Aldrich, Germany), Hydrochloric acid (BDR), aqueous 0.1M, Sodium hydroxide (BDR), aqueous '0.1M and 6.6M; O-phosphoric acid, Potassium Chloride, Potassium Monobasic Phosphate (Adwic) and Sulfuric acid (BDR), aqueous 5.0 M.

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

2.2. Instruments

A double-beam Jasco (Japan) UV/Visible spectrophotometer model J-760, connected to ACER compatible computer and a LaserJet printer is used. The bundled software is spectra manager Jasco (J-760) Version-2. The spectral bandwidth is 0.2 nm and the wavelength scanning speed was 1000.0 $nm.min^{-1}$. The absorption spectra of the reference and the test solutions are recorded in 2.0-mL quartz cells at 25.0 ^0C, using ‘$\Delta\lambda$ = 4 nm and scaling factor of 10 for computing first derivative (D^1).

A spectrofluorimeter (BIO-TEK Kontron, Switzerland) Model SFM25 connected to IBM compatible PC. The Bundled software was WIND25 personal spectroscopy software. The excitation and

emission spectra were recorded over the range of 200 – 800 nm at room temperature.

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 studied drugs

For spectrophotometric technique, stock standard solutions of (I), (II) and (III), each having concentration of (0.5 $mg.ml^{-1}$) were prepared respectively in 0.1M HCl, methanol and phosphate buffer pH 7, used as working standard solutions. While, stock standard solutions of (I), (II) and (III), each having concentration of (0.1 $mg.ml^{-1}$) were prepared respectively in methanol, used as working standard solutions for spectrofluorimetric technique.

2.3.2. Standard solution of Hydrochlorothiazide:

Stock standard solution of HCTZ having concentration of 0.5 $mg.ml^{-1}$ was prepared in methanol, and used as a working standard solution.

2.3.3. Standard solution of degradates

2.3.3.1. Standard solution of Trosemide acid-degradate

Standard solution of (I) acid-degradates was prepared by mixing 50 mg of authentic (I) with 10 ml 5M sulfuric acid, refluxing for 12.0 hours, cooling, neutralizing with the media with 6.6M sodium hydroxide, and increasing the volume to 100 ml with 0.1M HCl to obtain a concentration of 0.5 $mg.ml^{-1}$.

2.3.3.2. Standard solution of olmesartan alkaline-degradate

Standard solution of (III) alkaline-degradate was prepared by mixing 50 mg of authentic (III) with 10 ml 0.1M sodium hydroxide, refluxing for 20.0 minutes, cooling, neutralizing with the media with 0.1M HCl, and raising the volume to 100 ml with phosphate buffer pH 7 to obtain a concentration of 0.5 $mg.ml^{-1}$.

Complete degradation is checked by TLC using silica gel 60 F254 plates and chloroform: ethyl acetate: methanol [8.0: 8.0: 4.0] as a developing system.

2-4. Procedures:

2-4.1.Spectrophotometric technique:

2-4.1.1. Determination of Trosemide:

First derivative of ratio spectra method (DR^1):

Calibration curve was performed by transferring aliquots of (I) working standard solution into a series of 25 ml volumetric flasks, and diluting to volume with 0.1M HCl to obtain a concentration range of 2–40 $\mu g.ml^{-1}$. The spectrum of acid-degradate solution having concentration 2.0 $\mu g.ml^{-1}$ was scanned and stored in the instrument PC as a devisor. The spectra of (I) were divided by the devisor's spectrum, then the first derivative of the ratio spectra (DR^1) were computed at 272.00 nm, plotted versus concentrations, and the regression equation was computed.

2-4.1.2. Determination of Irbesartan:

First derivative of ratio subtraction spectral method:

The overlapping spectra of a binary mixture, (II) with hydrochlorothiazide (HCTZ) were resolved by adopting the ratio subtraction technique. The spectra of (II) working standard solutions were scanned from 200–400 nm and stored in the computer. The spectra of the laboratory-prepared mixtures were divided (absorbance at each wavelength) by the spectrum of 10.0 $\mu g\ ml^{-1}$ of (HCTZ). The absorbance in the plateau region was subtracted at wavelength above 305 nm (the constant). The obtained curves were multiplied (absorbance at each wavelength) by the spectrum of 10.0 $\mu g\ ml^{-1}$ of (HCTZ). Then the first derivative of the ratio subtraction was computed at 262.00 nm, plotted versus concentrations, and the regression equation was computed.

2-4.1.3. Determination of Olmesartan:

2-4.1.3.1.A. First derivative of ratio spectra method (DR^1):

Into a series of 25 ml volumetric flasks aliquots of (III) working standard solution were transferred, and the volume was then diluted with phosphate buffer pH 7 to obtain a concentration range of 2–50 $\mu g.ml^{-1}$. The spectrum of alkaline-degradate solution having concentration 2.0 $\mu g.ml^{-1}$ was scanned and stored in the instrument PC as a devisor. The spectra of (III) were divided by the devisor's spectrum, then the first derivative of the ratio spectra (DR^1) were computed at 278.00 nm, the calibration curve was then plotted versus concentrations, and the regression equation was computed.

2-4.1.3.1.B. First derivative of ratio subtraction spectral method:

The spectra of (III) working standard solutions were scanned from 200–400 nm and stored in the computer. The spectra of the laboratory-prepared mixtures were divided (absorbance at each wavelength) by the spectrum of 5.0 $\mu g\ ml^{-1}$ of (HCTZ). The absorbance in the plateau region was subtracted at wavelength above 305 nm (the constant). The obtained curves were multiplied (absorbance at each wavelength) by the spectrum of 5.0 $\mu g\ ml^{-1}$ of (HCTZ). Then the first derivative of the ratio subtraction was computed at 268.00 nm, plotted versus concentrations, and the regression equation was computed, to resolve the overlapping present between the spectra of (III) and hydrochlorothiazide (HCTZ) binary mixture.

2-4.1.3.1.C. First derivative of pH-induced difference spectrophotometric method (DD^1):

Aliquots of (III) working standard solution were transferred into two sets of 25 ml volumetric flasks, diluted with borate buffer pH 8.0 in the first set and with 0.1M NaOH pH 13.0 in the second set, to obtain a concentration range of 2-40 $\mu g.ml^{-1}$. The absorption spectra of the first set were scanned against borate buffer pH 8.0 and the second set against 0.1M NaOH pH 13.0. The differences in the absorption spectra (ΔA) were determined and the first derivative of ΔA spectra (DD^1) was then computed. The calibration curve was constructed by plotting the amplitudes at 256.00 nm versus concentrations, and the regression equation was then computed.

2-4.2. Spectrofluorimetric technique:

This technique affords a higher sensitivity, if compared with those spectrophotometric and chromatographic ones, where it permits the determination of the examined substances in a concentration reaches to one part per trillion[60,61]. In this method, each of (I), (II) and (III) investigated drugs can be determined with a higher sensitivity.

2-4.2.1. Determination of Trosemide:

Aliquots equivalent to 0.3–1.5 ml of (I) working standard solution were transferred into 100.0 ml volumetric flasks and the volume was completed to the mark with 0.1M hydrochloric acid, to give a concentration of 300–1500 $ng.ml^{-1}$. The fluorescence intensity was recorded at $\lambda_{emission}$ 407 nm using $\lambda_{excitation}$ at 237 nm. The calibration graph was plotted representing the relationship between emission intensity against concentrations and the regression equation was computed.

2-4.2.2. Determination of Irbesartan:

The calibration curve was performed by transferring aliquots of (II) working standard solution into a series of 100 ml volumetric flasks, and diluting with water to obtain a concentration range of 300–2300 $ng.ml^{-1}$. The fluorescence intensity was recorded at $\lambda_{emission}$ 390 nm using $\lambda_{excitation}$ at 224 nm, which then plotted versus concentrations, and the regression equation was computed.

2-4.2.3. Determination of Olmesartan:

0.03–0.2 ml of (III) working standard solution was transferred into 100.0 ml volumetric flasks and the volume was completed to the mark with 0.1M hydrochloric acid, to give a concentration of 30–200 $ng.ml^{-1}$. The fluorescence intensity was recorded at $\lambda_{emission}$ 409 nm using $\lambda_{excitation}$ at 221 nm. The calibration graph was plotted representing the relationship between emission intensity against concentrations and the regression equation was computed.

2-4.3. Assay of the pharmaceutical preparations:

For spectrophotometric technique, twenty tablets of Examide®, Co-Approval®, Erastapex® and Erastapex plus® were individually weighed to get the average weight of the tablets and finely powdered, respectively. A sample of the powdered tablets, claimed to contain '50 mg' and '30 mg' of '(I) and (III)' and '(II) and (III)', was transferred separately to 100 ml volumetric flasks, dissolved in 50 ml of '0.1M HCl and methanol' for (I) and '(II) and (III)', filtered and then the volume was brought to 100 ml with the same solvents. Also, phosphate buffer pH 7 was used as a solvent for dissolving and diluting the powdered sample of (III) '50 mg' only, to be determined by adopting the derivative ratio technique. These prepared solutions were used as stock working solutions. While for spectrofluorimetric technique, a sample of the powdered tablets, claimed to contain '10 mg' of '(I), (II), and (III), were transferred separately to 100 ml volumetric flasks dissolved in 50 ml methanol, filtered and then the volume was brought to 100 ml with the same solvent to prepare stock working solutions. Then the mentioned procedure under 2.4., was utilized for both spectrophotometric and spectrofluorimetric methods.

2-4.4. Spectrofluorimetric Determination of Trosemide in plasma samples:

Into a 10 ml centrifuging-tube, aliquots equivalent to 20 and 30 µg of (I) working standard solution were transferred, followed by 1 ml of human plasma and vortexed for 20 second. Then 1.5 ml of acetonitrile was added to precipitate the proteins, vortexed for 30 second, followed by addition of 2 ml methanol, vortexed again for 1 min and then centrifuged at 3000 rpm for 20 min. The supernatant was transferred to 25-ml volumetric flask, evaporated to dryness at 70^0C under vacuum, then the residue was re-constituted with the least amount of methanol, vortexed for 20 second, and completed to the mark by 0.1M HCl. Then the relative fluorescence for each concentration was recorded, and the concentration was calculated from the regression equation. All the steps in this application was adopted according to CAROLINA et al[62].

3. Results and Discussion:

3.1.Spectrophotometric methods

The absorption spectra of (I) and (III) and their degradation products shown in (Figures 2a-2b), exhibit severe overlapping that prevents the use of direct spectrophotometric determination of each drug in presence of its degradate. So, derivative ratio was

utilized for determination of both investigated drugs in presence of their degradates. Also, pH-induced difference spectrophotometric technique was adopted for the determination of (III) in presence of its alkaline-degradate. The proposed scheme for degradation of (I) and (III) is shown in (Figures 3a-3b), where Fourier transform infrared "FT-IR" and mass spectrometry "MS" were used for explaining the degradation behavior of I and III.

The selection of the optimum wavelength was based on the fact that the absolute value of the total derivative spectrum at the selected wavelength has the best linear response to the analyte concentration. It is not affected by the concentration of any other component and gives a near-zero intercept on the ordinate axis of the calibration curve. Therefore, 272.0 nm and 278.0 nm were chosen as optimum working wavelengths for the determination of (I) and (III) in presence of their degradates by utilizing the proposed method, as shown in (Figures 4a-4b) respectively. Also 256.0 nm was used for determination of (III) in presence of its alkaline degradates by computing the first derivative of pH-induced difference spectrophotometry, as shown in (Figure 4c).

On the other hand, recently (II) and (III) were used separately in combination with HCTZ as antihypertensive drugs. Unfortunately, trails to determine either (II) or (III) in presence of HCTZ were not recorded, regarding to severe overlapping obtained in the absorption zero-order UV spectra of (II) and (III) and HCTZ, separately, as shown in (Figures5a-5-b). This extensive overlapping of the spectral bands of the two allowing us to utilized ratio subtraction technique, where 262.0 nm and 268.0 nm were selected as optimum working wavelengths for the determination of (II) and (III) in presence of HCTZ, as shown in (Figures 6a-6b) respectively.

3.2. Spectrofluorimetric method

A native strong fluorescence was observed upon dissolving '(I) and (III)' and (II) in 0.1M Hydrochloric acid and water, where these two solvents were selected among different solvents, including 0.1M hydrochloric acid, 0.1M sodium hydroxide, methanol and distilled water and the best emission intensity was obtained on using the last mentioned selected ones as dilution solvents for '(I) and (III)' and (II), as shown in (Figures 7a-7c), respectively. Scanning the emission spectra for the studied investigated drugs showed $\lambda_{emission}$ 407.0 nm, 409.0 nm and 390.0 nm, using $\lambda_{excitation}$ at 237.0 nm, 221.0 nm and 224.0 nm, attributing to the high conjugation, as shown in (Figures 8a-8c), respectively.

The adopted spectrofluorimetric method is highly sensitive, allowing us to determine (I) in plasma, where the recovery was found to be 92 %. In this application, different solvents were used to precipitate proteins including 6M hydrochloric acid [63], methanol [64] and acetonitrile[65, 66], but the best results were obtained in using acetonitrile, regarding to the disadvantage of methanol where incomplete precipitation of the plasma proteins was obtained, also abnormal brown color, which could be explained by the instability of the drug in acid media[67] in using hydrochloric acid. Consequently, the proposed precipitation and extraction method explained earlier was used[62].

3.3. Methods validation.

ICH-guidelines[4] for the methods of validation were followed, where all the validation parameters are shown in (Table 1).

3.3.1. Linearity:

A linear correlation was obtained between 'peak amplitude and/or fluorescence intensity' and concentration of the investigated drugs "I, II and III" in a range of '2-40 μgml^{-1}, 2-50 μgml^{-1} and (2-50, 2-40, 2-50 μgml^{-1})' with [correlation coefficient [r] = 0.9998, 0.9998 and '0.9998, 0.9997, 0.9997'] and '300–1500 ng ml^{-1}, 300–2300 $ngml^{-1}$ and 30–200 $ngml^{-1}$' with [correlation coefficient (r) = 0.9997, 0.9998 and 0.9998] for the spectrophotometric and spectrofluoremetric determinations, respectively.

3.3.2. Accuracy:

The 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 methods were statistically compared with those results obtained by the reference methods [68-70]. 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 [71].

3.3.3. Repeatability and reproducibility:

The intra- and inter-day precision was evaluated by assaying freshly prepared solutions in triplicate.

3.3.4. Specificity:

I and III were determined in solutions of laboratory prepared mixtures containing their acid and alkaline-degradates, also II and III could be determined in presence of HCTZ by the proposed

methods. The Recovery % and S.D. proved the high specificity of these methods, as shown in (Table 2).

3-4. Standard addition technique:

The proposed methods were applied for the determination of the studied drugs in the pharmaceutical preparations. The results 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 each pure drug. The results of the commercial tablets analysis and the standard addition method (recovery study) of [I, II and III] are shown in (Tables 3-5) suggested that there is no interference from any excipients, which are normally present in tablets.

Also, the proposed adopted spectrofluorimetric method could be successfully applied for determination of I in spiked human plasma samples by liquid-liquid extraction technique, where the recovery was found to be 92 %, as shown in (Table 6).

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

3.5.1. 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 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 9a-9d).

3.5.2. 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 10a-10d).

4- Conclusion:

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

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

Parameters	Torasemide		Irbesartan		Olmesartan			
	Derivative Ratio	Spectrofluor-imetry	Ratio Subtraction	Spectrofluor-imetry	Derivative Ratio	Difference Spectrophotometry	Ratio Subtraction	Spectroflu-orimetry
	μgml^{-1}	$ngml^{-1}$	μgml^{-1}	$ngml^{-1}$	μgml^{-1}			$ngml^{-1}$
Linearity	2-40	300-1500	2-50	300-2300	2-50	2-40	2-50	30-200
Intercept	0.2682	0.0602	-0.001	0.4182	-0.0108	-0.0005	0.0293	-0.3923
Slope (b)[a]	0.2569	0.0685	0.0013	0.0452	0.0167	0.0012	0.001	0.537
Correlation Coefficient (r)	0.9998	0.9997	0.9998	0.9998	0.9998	0.9997	0.9997	0.9998
Accuracy[b]	100±0.57	100.23±0.9	100.02±0.92	100.86±1.24	100.08±0.73	99.95±0.79	101±0.47	99.91±1.12
Precision								
Repeatability[b]	104±0.57	99.6±0.47	99.6±0.45	99.8±0.66	100.2±0.45	100.2±0.25	99.8±0.48	100.1±0.65
Intermediate Precision[b]	99.8±0.64	99.8±0.63	99.5±0.68	99.6±0.78	99.8±0.74	100.4±0.61	100.1±0.61	100.4±0.74

[a]Regression equation = "A = a + bc". [b]Mean ± S.D.

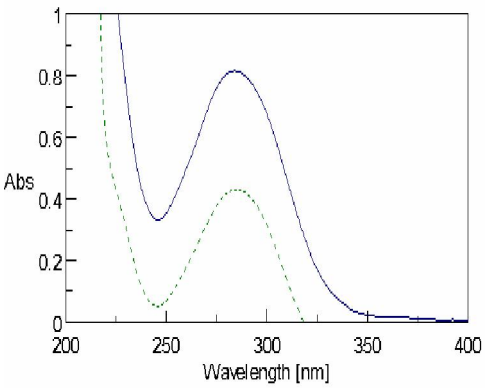

Figure (2-a): Zero-order absorption spectra of Torasemide (—) and its acid-degradate (...), (each of 20.0 μg ml^{-1}).

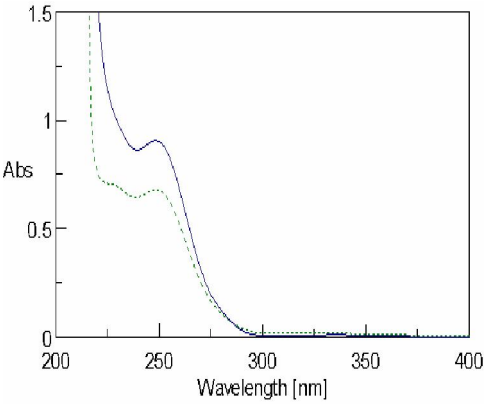

Figure (2-b): Zero-order absorption spectra of Olmesartan (—) and its alkaline-degradate (...), (each of 20.0 μg ml^{-1}).

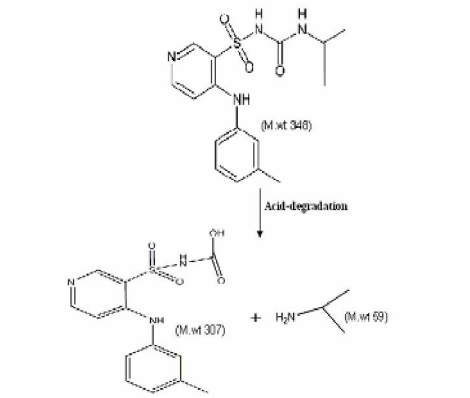

Figure (3-a): Suggested degradation pathway for Torasemide.

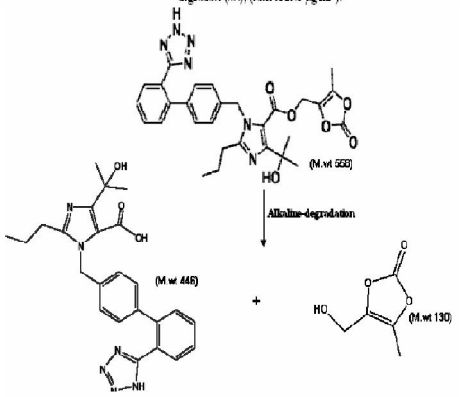

Figure (3-b): Suggested degradation pathway for Olmesartan.

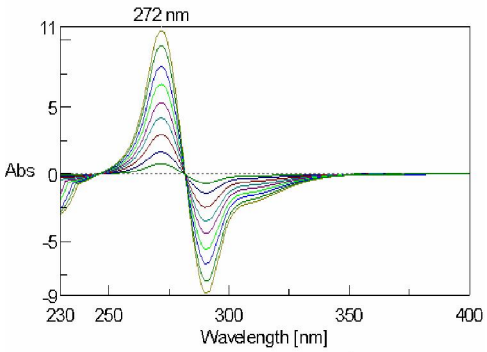

Figure (4-a) First-derivative of the ratio spectra (DR^1) for different concentrations (2.0-40.0 μg ml^{-1}) of Torasemide at 272.0 nm, using 2.0 μg ml^{-1} of its acid-degradate as a devisor.

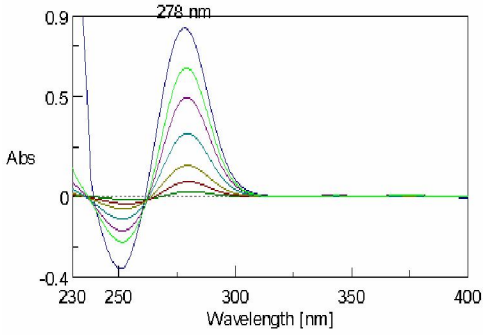

Figure (4-b): First-derivative of the ratio spectra (DR^1) for different concentrations (2.0-50.0 μg ml^{-1}) of Olmesartan at 278.0 nm, using 2.0 μg ml^{-1} of its alkaline-degradate as a devisor.

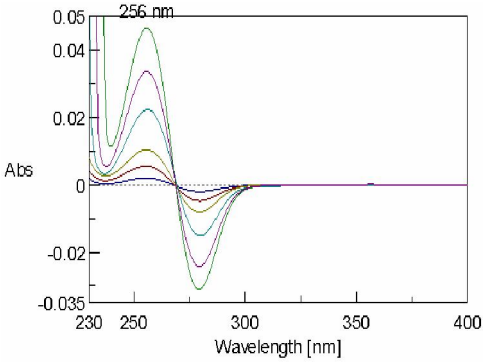

Figure (4-c): First derivative of difference spectra (DD^1) for different concentrations (2.0–40.0 $\mu g ml^{-1}$) of Olmesartan medoxomil at 256.0 nm.

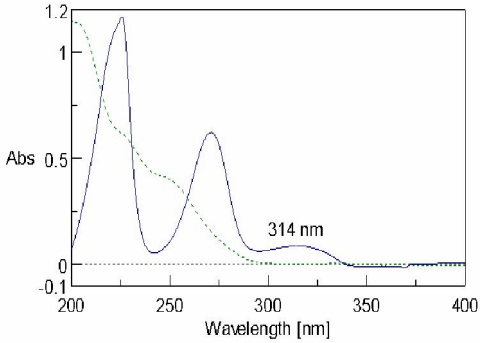

Figure (5-a): Zero-order absorption spectra of Irbesartan (....) and HCTZ (—), (each of 10 $\mu g ml^{-1}$).

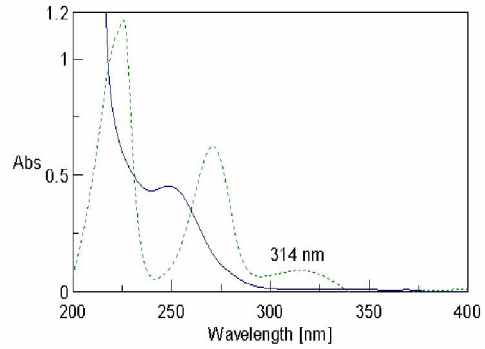

Figure (5-b) Zero-order absorption spectra of Olmesartan (—) and HCTZ (....), (each of 10 $\mu g ml^{-1}$).

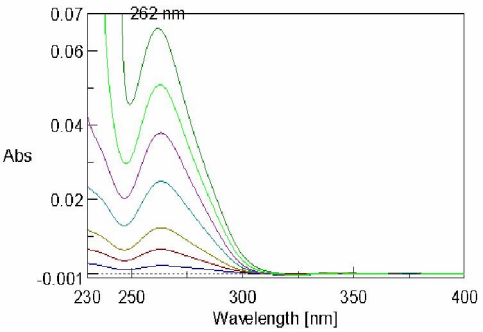

Figure (6-a): First order division spectra of laboratory-prepared mixtures of Irbesartan and HCTZ, at 262.0 nm, using 10 $\mu g ml^{-1}$ of HCTZ as the divisor.

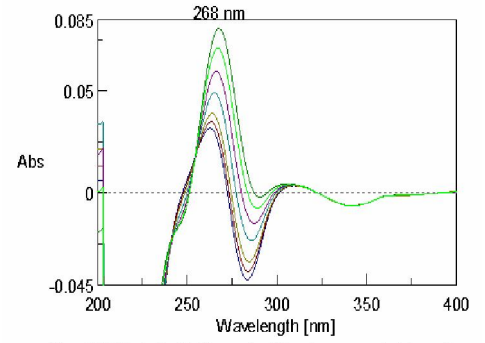

Figure (6-b): First order Division spectra of laboratory-prepared mixtures of Olmesartan and HCTZ, at 268.0 nm, using 5 $\mu g ml^{-1}$ of HCTZ as the divisor.

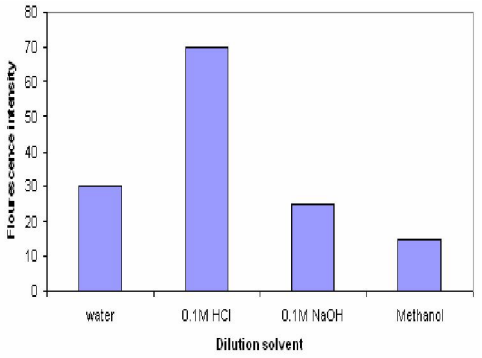

Figure (7-a): Effect of dilution solvent on fluorescence intensity of Torasemide in a concentration (1 $\mu g ml^{-1}$).

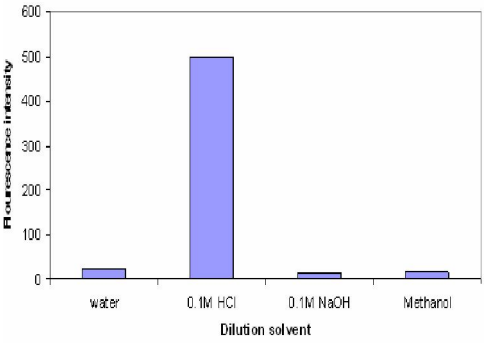

Figure (7-b): Effect of dilution solvents on fluorescence intensity of Olmesartan in a concentration (1 $\mu g ml^{-1}$).

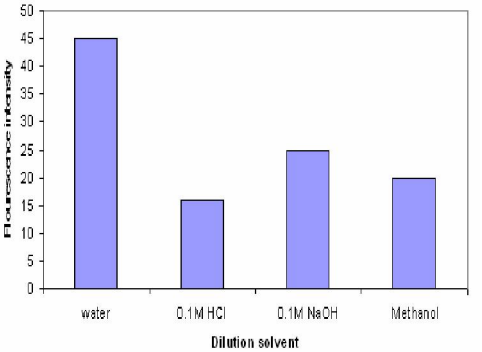

Figure (7-c): Effect of dilution solvents on the fluorescence intensity of Irbesartan in a concentration (1 $\mu g ml^{-1}$).

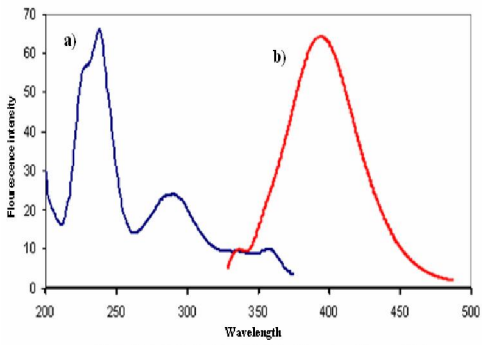

Figure (8-a): Excitation (a) and Emission (b) spectra of Torasemide in 0.1 M HCl

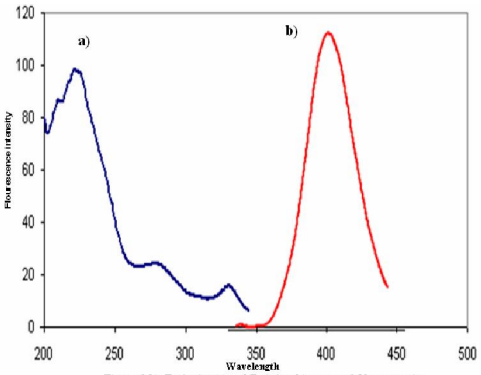

Figure (8-b): Excitation (a) and Emission (b) spectra of Olmesartan in 0.1M HCl

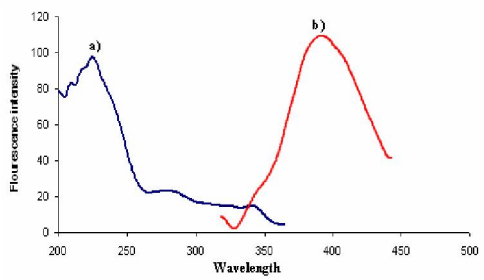

Figure (8-c): Excitation (a) and Emission (b) spectra of Irbesartan in Distilled water.

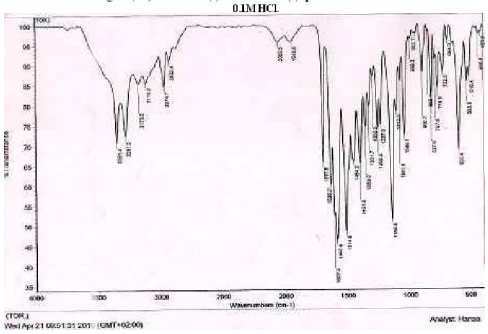

Figure (9-a): IR spectrum of the Intact Torasemide.

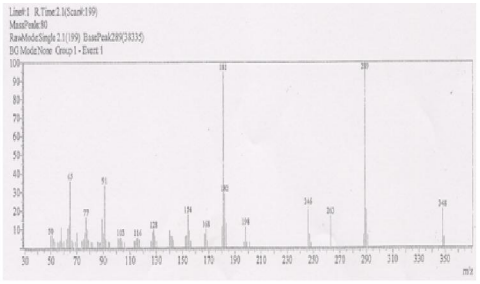

Figure (9-b): Mass spectrum of the intact Torasemide.

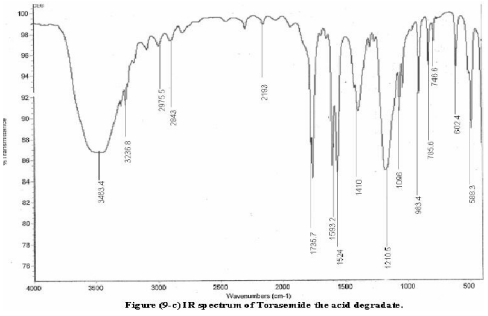

Figure (9-c) IR spectrum of Torasemide the acid degradate.

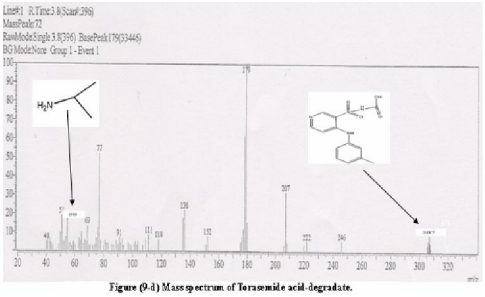

Figure (9-d) Mass spectrum of Torasemide acid-degradate.

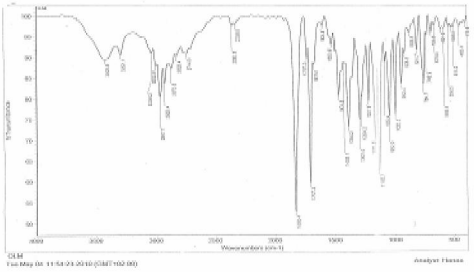

Figure (10-a): IR spectrum of the Intact Olmesartan.

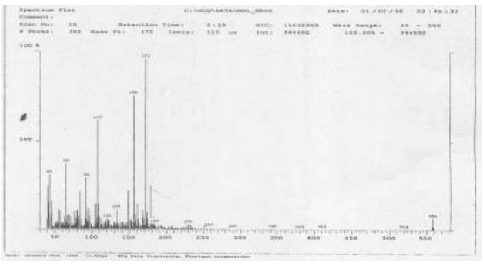

Figure (10-b): Mass spectrum of the intact Olmesartan.

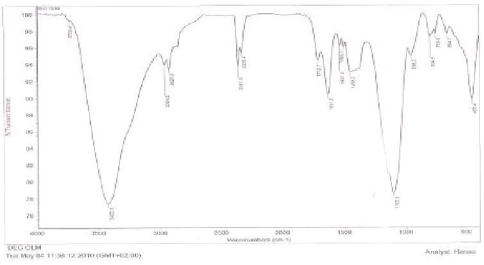

Figure (10-c): IR spectrum of Olmesartan alkaline degradate.

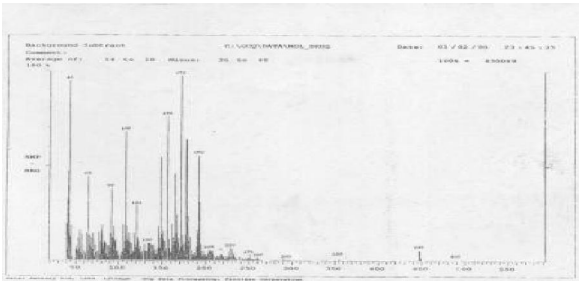

Figure (10-d): Mass spectrum of Olmesartan the alkaline degradate.

Table 2: Results for Torasemide (I), Irbesartan (II) and Olmesartan (III) in laboratory prepared mixtures by the proposed methods:

Sample No.	% of Interfering substance	Recovery%*						
		Torasemide	Irbesartan		Olmesartan			
		Derivative Ratio	Ratio Subtraction	Spectrofluor-imetry	Derivative Ratio	Difference Spectrophotometry	Ratio Subtraction	Spectroflu-orimetry
1	20	100.5	101.54	100.16	100.48	100.83	99.30	99.43
2	30	98.55	100.77	-	101.68	99.67	100.00	-
3	40	99.72	100.38	100.53	98.68	100.00	99.90	101.29
4	50	98.55	99.23	-	101.08	100.75	101.00	-
5	60	101.67	99.92	100.90	100.48	101.17	100.80	101.66
6	70	101.28	100.77	-	100.78	102.00	101.15	-
7	80	-	101.54	98.69	101.08	-	-	98.68
8	90	-	102.15	99.10	100.78	-	-	101.29
9	100	-	-	101.64	101.38	-	-	100.36
Mean		100.04	100.79	100.17	100.93	100.74	100.42	100.45
S.D.		1.34	0.95	1.11	0.31	0.83	0.82	1.19

*Mean of four determinations.

Table 3: Determination of Torasemide in pharmaceutical preparation[a] by the proposed {spectrophotometric and spectrofluorimetric} methods and application of standard addition technique.

Pharmaceutical Preparation	Claimed	% Found ± SD*		Standard addition technique							
		Derivative Ratio	Spectro-fluorimetry	Derivative Ratio	Spectro-fluorimetry	Pure added		Pure found		Recovery %*	
				Taken in μgml^{-1}	Taken in $ngml^{-1}$	Derivative Ratio in μgml^{-1}	Spectro-fluorimetry in $ngml^{-1}$	Derivative Ratio in μgml^{-1}	Spectro-fluorimetry in $ngml^{-1}$	Derivative Ratio	Spectro-fluorimetry
Examide ® tablets 20 mg B.N: MT1120410[a]	20 mg	99.2 ±0.54	101.5 ±0.46	10	300	2	50	1.996	50.60	99.80	101.20
						5	100	5	99.40	100.00	99.40
						15	150	15.075	149.780	100.50	99.80
						20	200	20.04	201.00	100.20	100.50
						25	250	24.925	253.00	99.70	101.20
						30	300	30.06	298.80	100.20	99.60
Mean ±S.D.										100.07 ±0.29	100.28 ±0.80

*Mean of four separate determinations.

Table 4: Determination of Irbesartan in pharmaceutical preparation[a] by the proposed {spectrophotometric and spectrofluorimetric} methods and application of standard addition technique.

Pharmaceutical Preparation	Claimed	% Found ± SD*		Standard addition technique							
		Ratio Subtraction	Spectro-fluorimetry	Ratio Subtraction	Spectro-fluorimetry	Pure added		Pure found		Recovery %*	
				Taken in μgml^{-1}	Taken in $ngml^{-1}$	Derivative Ratio in μgml^{-1}	Spectro-fluorimetry in $ngml^{-1}$	Derivative Ratio in μgml^{-1}	Spectro-fluorimetry in $ngml^{-1}$	Ratio Subtraction	Spectro-fluorimetry
Co-Approval® tablets 300mg/12.5mg B.N: 1145[a]	300 mg	100.20 ±0.55	98.50 ±0.47	10	300	2	50	2.002	49.90	100.10	99.80
						5	75	4.945	75.00	98.90	100.00
						10	100	9.960	100.50	99.60	100.50
						20	125	20.040	125.25	100.20	100.20
						30	150	30.030	149.55	100.10	99.70
						40	200	40.080	200.40	100.20	100.20
Mean ±S.D.										98.85 ±0.52	100.07 ±0.29

*Mean of four separate determinations.

Table 5-a: Determination of Olmesartan in pharmaceutical preparation[a] by the proposed derivative ratio and pH-induced difference spectrophotometric methods [DR^n and DD^n] and application of standard addition technique.

Pharmaceutical Preparation	Claimed	% Found ± SD*		Standard addition technique							
		DR^n	DD^n	Taken μgml^{-1}		Pure added μgml^{-1}		Pure found μgml^{-1}		Recovery %*	
				DR^n	DD^n	DR^n	DD^n	DR^n	DD^n	DR^n	DD^n
Erastapex® tablets 40 mg B.N: MT 3241009[a]	20 mg	100.1 0 ±0.42	100.50 ±0.52	10	10	5	5	4.99	5.01	99.80	100.20
						10	10	10.02	10.01	100.20	100.10
						20	15	19.84	14.97	99.20	99.80
						25	20	25.15	20.06	100.60	100.30
						35	25	35.035	25.30	100.10	101.20
						40	30	40.08	29.94	100.20	99.80
Mean ±S.D.										100.02 ±0.48	100.23 ±0.52

*Mean of four separate determinations.

Table 5-b: Determination of Olmesartan in pharmaceutical preparation[a] by the proposed {spectrophotometric and spectrofluorimetric} methods and application of standard addition technique.

Pharmaceutical Preparation	Claimed	% Found ± SD*		Standard addition technique							
		Ratio Subtraction	Spectro-fluorimetry	Ratio Subtraction	Spectro-fluorimetry	Pure added		Pure found		Recovery %*	
				Taken in μgml^{-1}	Taken in $ngml^{-1}$	Derivative Ratio in μgml^{-1}	Spectro-fluorimetry in $ngml^{-1}$	Derivative Ratio in μgml^{-1}	Spectro-fluorimetry in $ngml^{-1}$	Ratio Subtraction	Spectro-fluorimetry
Erastapex plus® tablets 40mg/12.5mg B.N: MT0280110[a]	40 mg	100.20 ±0.48	98.80 ±0.84	10	300	5	20	4.99	20.02	99.80	100.10
						10	30	10.00	29.67	100.00	98.90
						20	50	20.10	49.80	100.50	99.60
						25	60	25.05	60.12	100.20	100.20
						35	80	34.895	80.08	99.70	100.10
						40	100	40.08	100.20	100.20	100.20
Mean ±S.D.										100.07 ±0.29	99.85 ±0.52

*Mean of four separate determinations.

Table 6: Determination of Torasemide in spiked human plasma by the proposed spectrofluorimetric method.

Spiked concentration ($ngml^{-1}$)	Recovery % ± S.D*
800.00	92.27 ± 0.56
1200.00	92.31 ± 0.47

* The mean percentage recovery of 3-separate determinations.

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