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# Stability-Indicating Densitometric and Spectrophotometric Determination of Lansoprazole and Pantoprazole Sodium.

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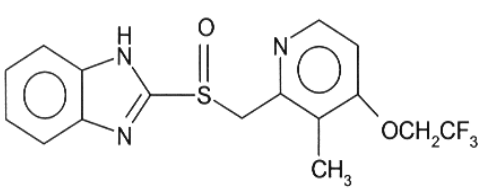
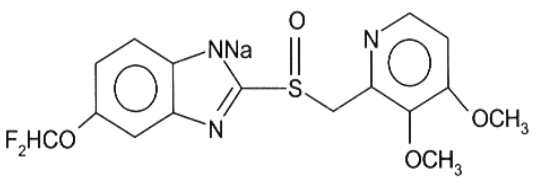
**Summary** Two procedures were presented for the selective determination of lansoprazole and pantoprazole sodium in presence of their degradates. Degradation was induced by incubating the pure drugs in borate buffer so1ution (pH 8) at 37°C for 5 days to yield their sulphenic acid and sulfenamide derivatives. The first procedure was based on the quantitative densitometric evaluation of thin layer chromatograms of lansoparzole at 285 nm and of pantoprazole sodium at 295 nm using sheets of silica gel 60 $F_{254}$ and a mobile phase of chloroform-methanol (10:0.6). Regression analysis of Beer's plots showed good correlations (r = 0.9998 and 0.9994) in concentration ranges of 1-9 µg / spot and 0.5-4.0 µg/spot for the two drugs, respectively. The procedure proved its selectivity in presence of up to 80% and 90[1]% of the two degraded drugs, respectively. The second procedure involved first derivative spectrophotometric measurement of lansoprazole in methanol at 297 nm. This procedure is sensitive in concentration range of 5-25 µg $ml^{-1}$ of the drug in presence of up to 70% of its degradates. The mean recoveries for pharmaceutical formulations were 99.1-100.3% and were in accordance with those obtained by compendial methods.

## Introduction

Lansoprazole (LZ) and pantoprazole sodium (PT-Na) are α-pyridinyl methyl-sulfinylbenzimidazoles; the most effective suppressors of gastric acid secretion through specific inhibition of the gastric $H^+$, $K^+$ ATP ase enzyme system at the gastric parietal cell[(1-3)] only Lansoprazole is official in USP[(4)]. The literature survey reveals spetrophotometric[(59)] electrochemica1[(10,11)] and electrophoretic[(12-13)] methods for the determination of the two cited drugs. In addition to some TLC[(14,15)] and HPLC[(14,16-18)] methods.



The cited benzimidazole drugs are sensitive compounds which can be easily degraded, hence are dispensed as enteric coated formulas. Thus the aim of the present study is to develop stability-indicating methods for the determination of LZ and (PT-Na) using densitometry and first derivative ($^1$D) spectrophotometry.

## Experimental

### Instrumentation

A Camag TLC Scanner III (Switzerland) with CATS 4 Computer software. The samples were applied to the plates using Hamilton syringe l0-µL capacity. Aluminum TLC plates (2O×2Ocm) precoated with silica gel 60 $GF_{254}$ were purchased from E-Merck (Darmstadt, Germany).

Shimadzu UV-visible spectrophotometer 1601, Lutron Digital MV-pH meter and Vector IR Spectrophotometer, 8201 PC.

### Materials and reagents

All chemicals were of analytical grade and solvents were of spectroscopic grade. Methanol (Fischer, England), ethanol absolute (Riedell-detlean, Germany), chloroform (Prolabo) HC1 (Riedell—detlean, Germany) were used. Borate buffer pH 8 was prepared by mixing 0.2 M boric acid (E1-Gomhouria, Egypt) with 0.05 M borax (E1-Gomhouria, Egypt).

Pure Lansoprazole (LZ) certified to contain 99.95% (sigma, Germany) and pure pantoprazole sodium sesquihydrate (PT-Na), certified to contain 99.93% (Byk, Golden, Konstanz, Germany) were used. Lansor capsules labeled to contain 15 mg lansoprazole (Hoechst Marion Roussel S.A.E) controloc tablets labeled to contain 40 mg pantoprazole (Byk, Konstanz, Germany) and pantoloc tablets labeled to contain 20 mg pantoprazole as pantoprazole sodium sesquihydrate (Medical union pharmaceuticals, Ismailia, Egypt) were purchased from local market.

Stock solutions (1mg $m1^{-1}$) of LZ and PT-Na were prepared in methanol and ethanol, respectively. A standard solution of Lanzoprazole (0.1 mg $ml^{-1}$) was prepared by diluting its stock solution with methanol. These solutions are stable for 8 hours at room temperature and for one week in refrigerator.

**Preparation of Degraded samples**

Pure LZ or PT-Na (50 mg), were dissolved in the least amount of methanol or water, respectively then diluted to 100 ml with borate buffer (pH 8) and incubated at 37°C for 5 days. Evaporation of each solution to dryness was done under vacuum then extraction three times, each with 15 ml methanol for LZ or ethanol for PT-Na was performed. The extracts were filtered into a 50 ml-volumetric flask and completed to volume with the corresponding solvent. This solution assumed to contain the sulfenamide and sulfenic acid degradates derived from 1 mg $m1^{-1}$ pure LZ or PT-Na, used for the densitometric procedure for each drug. The previously prepared methanolic solution of degraded LZ was diluted with methanol so as each 1 ml contain the two degradates derived from 0.1mg of pure LZ to be used for $^1D$ spectrophotometry.

**Calibration**

Densitometry-Aliquot portions (1-9 ml) or (0.5-4.0 ml) of stock solution of LZ or PT-Na, respectively were transferred to a separate series of 10-ml volumetric flasks and completed to volume with methanol or ethanol, respectively. Ten microlitres of each solution was applied to the TLC plate using a Hamilton micro-syringe and the plate was developed by ascending way with chloroform—methanol (10:0.6 v/v) to a distance of 16cm, air dried and detected under UV lamp at 254 nm. Densitometric scanning was done at 285 nm and 295 nm for LZ and PT-Na, respectively under the following instrumental condition:

- Photomode: Reflection.
- Peak threashold area: 100.
- Scan mode: Zigzag.
- Peak threashold, slope: 5.
- Swing width: 10mm

The peak areas were integrated and related to drug concentrations to obtain the calibration curves.

$^1D$ spectrophotometry-Volumes (0.5-2.5 ml) of methanolic standard LZ solution (0.1 mg $ml^{-1}$) were introduced into a series of 10ml volumetric flasks and diluted to volume with methanol. The first derivative spectrum ($^1D$) of each solution was recorded and trough at 297 nm was measured in cm at $\Delta\lambda$ =2 and ordinate

values of (+0.02 to -0.06). A calibration curve was constructed by relating trough heights at 297 nm to drug concentrations in μg $m^{-1}$.

**Mixtures of pure drugs with their degradates**

Densitometry-Aliquot volumes of stock standard methanolic solution of LZ equivalent to 8.1-1.8 mg of drug were introduced into a series of 10 ml volumetric flasks containing volumes of degraded solution equivalent (0.9-7.2 mg) of LZ degradates (prepared as detailed under preparation of degraded samples. The flasks were completed to volume with methanol. This series contained 10-80 % of degraded LZ was analysed as detailed above.

For PT-Na the above procedure was followed by mixing aliquots of stock PT-Na in ethanol equivalent to 4.0-0.5 mg intact drug with volumes of its degraded solution corresponding to the degradates derived from 1.0-4.5 mg PT-Na and completed to 10 ml with ethanol, these solutions containing 20-90% of degraded PT-Na were analysed by densitometry as described under calibration. The concentration of each pure drug was calculated from the corresponding regression equation

$^1$D spectrophotometry-Into a series of 10 ml volumetric flasks, volumes(1.8-0.6 ml)of standard LZ solution in methanol(0.1 mg $m1^{-1}$) containing 0.18-0.06 mg drug, were mixed with volumes (0.2-1.4 ml) of its degraded solution containing degradates derived from 0.02-0.14 mg LZ. The volumes were completed to 10 ml with methanol to obtain solutions containing 10-70% degraded LZ. The above detailed procedure for $^1$D spectrophotometry was followed.

**Pharmaceutical formulations**

The contents of ten lansor capsules or ten controloc or pantoloc tablets were weighed and finelly ground. A weighed amount of the fine powder equivalent to 50 mg of pure drug were transferred to a 50-ml beaker and extracted with (3 × 15 ml) methanol or ethanol for LZ or PT-Na, respectively by sonication for 30 min. The extracts were filtered into a 50-ml volumetric flask and completed to volume with appropriate solvent to obtain a clear filtrate claimed to contain 1 mg $m1^{-1}$ of each drug. Each filtrate was analysed by the proposed densitometric procedure as

described above. The content of each drug preparation was calculated by referring to the corresponding regression equation

For $^{1}$D spectrophotometry, the clear methanolic filtrate labeled to contain 1 mg m1$^{-1}$ LZ was diluted with methanol to obtain a solution assumed to contain 0.1 mg ml$^{-1}$ of pure drug. Then the later solution was analysed as detailed above and the drug content was calculated from the corresponding regression equation.

## Results and discussion

The problem of overcoming irrelevant interferences from additives, flavours or degradation products in pharmaceutical formulations will remain an everlasting problem for chemists. In this work, the degradation of two sensitive antiulcerative drugs, lansoprazole (LZ) and pantoprazole sodium PT-Na was accelerated in weak alkaline medium to find out selective procedures for the determination of pure drugs in presence of their degradates.

### Degradation of LZ and PT-Na

Upon trying degradation of both drugs using 0.1 N HCI, two degradates were obtained sulfenic acid and sulfenamide derivatives (Fig. 1). However, although degradation was rapid, yet the sulfenic acid has been found to be very unstable, undergoing further degradation within minutes. Thus analogues to omeprazole[(9)] degradation of LZ and PT-Na were investigated by incubating their solutions in borate buffer of pH 8 at 37°C. Complete degradation was found to be affected after 5 days yielding two degradates for each drug; sulfenamide and sulfenic acid derivatives.

The degradation of both drugs was confirmed by TLC and IR. The buffered solutions at pH 8 after complete degradation were evaporated to dryness under vacuum and extracted with methanol or ethanol for LZ or PT-Na, respectively. Extracts were tested by TLC on silica gel 60 $GF_{254}$ plates (20×20 cm) using a mobile phase of $CHCl_3$-$CH_3OH$ (1 0-0.6v/v) for both drugs. Two spots were obtained at $R_f$ 0.21 and 0.87 corresponding to sulfenic acid and sulfenamide derivatives of LZ,

whereas for the reference drug the $R_f$ was at 0.5. Similarly the $R_f$ values of the two degradation products of PT-Na were as at 0.22 and 0.72 and
that of pure drug was at 0.59.

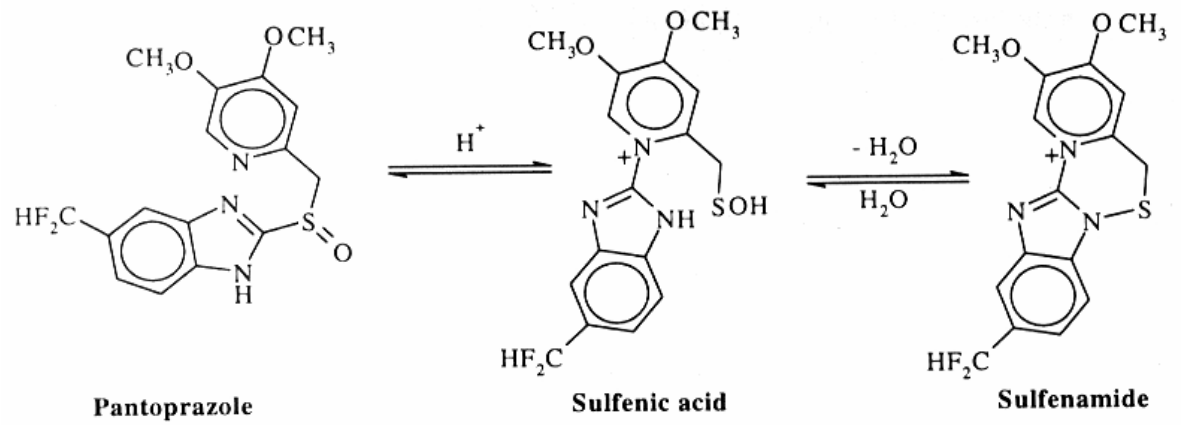



Fig 1. **Degradation pathway of pantoprazole**

The degradates of both drugs was purified on preparative plates using the same chromatographic system, extracted and evaporated to dryness, then confirmed by IR using KBr discs technique. The IR spectra of intact drugs showed principle absorption bands due to -NH, -C=C- , -C=N- and — S=O at 3233, 1582, 1454 and 1170 $cm^{-1}$ for LZ and at 3510, 1589, 1492 and 1165 $cm^{-1}$ for PT —Na[(1)]. However, the IR spectra of sulfenic acid degradate of both drugs showed broad bands around 3500-3200 $cm^{-1}$ due to overlapping of -NH- and -OH groups and disappearance of the peaks at 1200-1100 $cm^{-1}$ due to removal of sulfoxide (-S = O) stretching band[(20)]. In addition, the IR spectra of sulfenamide derivatives of the two drugs showed the removal of -NH and -S=O groups as confirmed by the disappearance of the absorption bands around 3200-3500 $cm^{-1}$ and 1200-1100 $cm^{-1}$ of the reference drugs.

Densitometric Procedure - Experimental conditions such as mobile phase, wavelength of scanning and swing width were optimized to provide accurate, presice and reproducible results for the determination of LZ and PT-Na in presence of their degradation products. The chosen swing width was 10 mm and wavelength of scanning was 285 nm for LZ and 295 nm for PT-Na. Various developing systems were tried to separate each drug from its degradates such as ethanol-hexane, n-butanol-ammonia-$H_2O$, methanol-acetonitrile and chloroform-methanol. The greatest differences between $R_f$ values of each drug and its degradation products

with minimum tailing were obtained upon using a mobile place consisting of chloroform-methanol in a ratio of 10: 0.6 v/v.

The specificity of the densitometric procedure was obvious in Figs. (2 and 3) where complete separation of each of LZ and PT-Na from their sulfenic acid and sulfenamide derivatives was noticed. It is noteworthy to mention that sulphenic acid degradate of each drug appears in the chromatogram in the form of two close peaks which suggested its presence in two forms at equilibrium with each other.

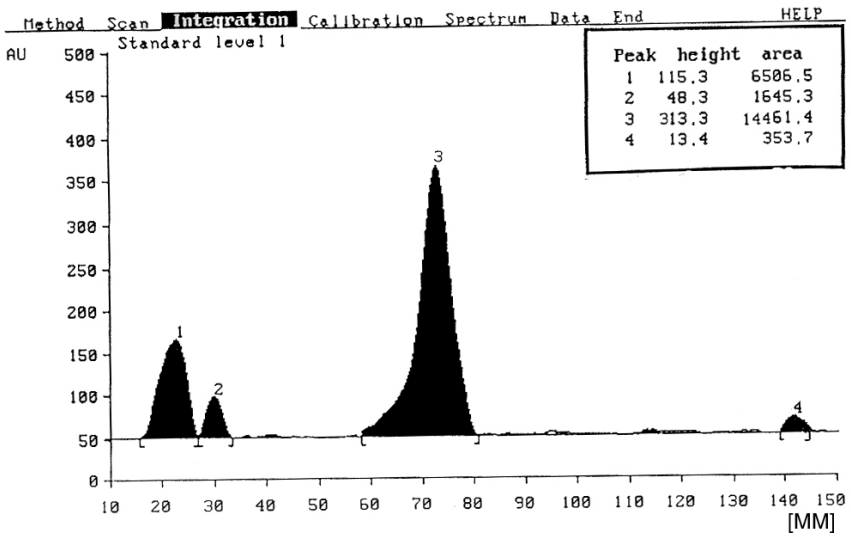



Fig. 2. Densitogram of laboratory prepared mixture of lansoprazole (3), 3.6 μg/spot, its sulfenic acid (1, 2) and its sulfenamide (4) degradates both derived from 5.4 μg/spot lansoprazole.

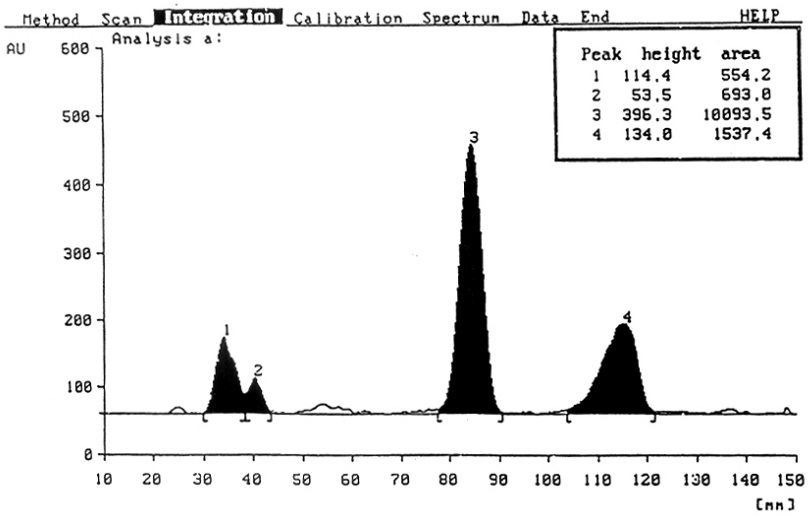



Fig. 3. Densitogram of laboratory prepared mixture of pantoprazole sodium (2.5 μg/spot) (3), its sulfenic acid degradate (1,2) and its sulfonamide degradate (4); both derived from 2.5 μg/spot pantoprazole sodium.

$^1$D spectrophotometic procedure has been considered the best resort for resolution of overlapped peaks. The UV absorption spectra of LZ and its two degradates in methanol showed marked band overlappings (Fig. 4) which disengaged in their first derivative curves (Fig. 5). As illustrated from the latter figure, intact drug has a typical trough at 297 nm at which its two degradates show minimum contribution, allowing for the possibility of the selective determination of intact LZ in presence of its degradation products using $^1$D-spectrophotometry at 297 nm. It should be pointed out that this method has been applied for the selective determination of intact PT-Na in a previous paper[9].

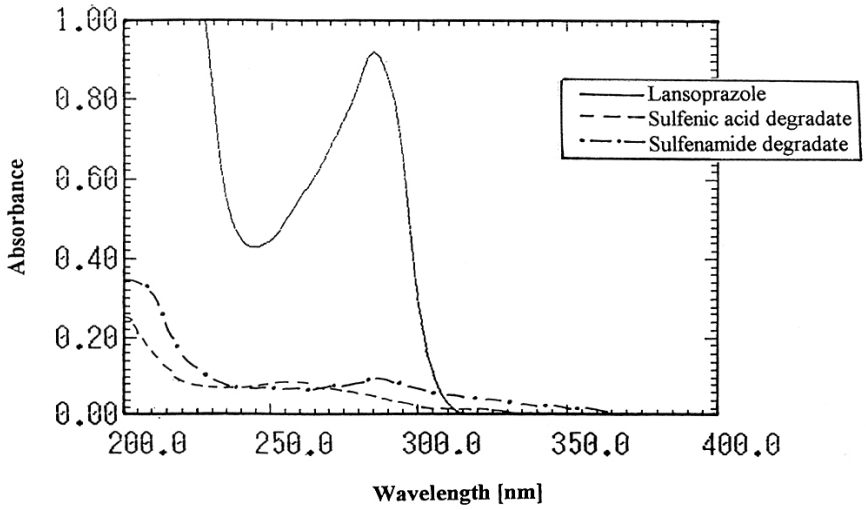



Fig. 4. Zero order spectra of intact lansoprazole (20 μg ml$^{-1}$), its sulfenic acid and sulfonamide degradates in methanol.

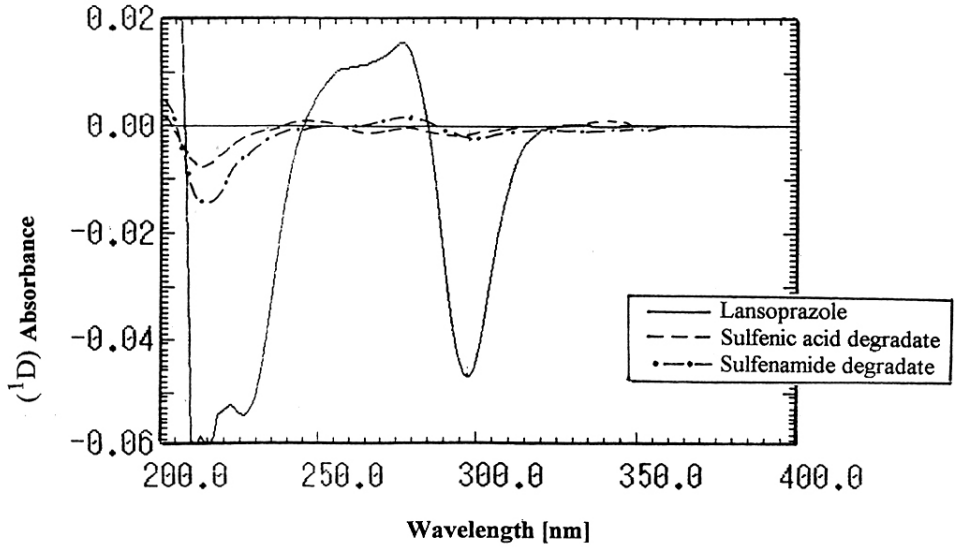



Fig. 5. First derivative spectra of intact lansorprazole (20 μg ml$^{-1}$), its sulfenic acid and sulfonamide degradates in methanol.

**Validation of the procedures**

The linearity range of the proposed procedures was found to obey Beer's law in the range of 1-9 µg/spot and 5-25 µg $m1^{-1}$ of LZ for densitometry and $^{1}D$ spectrophotometry, respectively. While for PT-Na, it was in the range of 0.5-4 µg/spot, for densitometry and good linearity was validated by the high value of the correlation coefficient (r = 0.9994-0.9999), table (1). Also the regression parameters were summarized in the same table.

**Table (1):** The analytical characteristics parameters for the regression equations, accuracy and precision of proposed densitometric and ($^{1}D$) Spectrophotometric Procedures

| Parameter | Densitometric procedure | | ($^{1}D$) Spectrophotometric procedure |
|---|---|---|---|
| | Lansoprazole | Pantoprazole | Lansoprazole |
| $\lambda_{max}$ nm | 285 | 295 | 297 |
| Linearity range | 1-9 µg/spot | 0.5- 4.0 µg/spot | 5-25 µg $ml^{-1}$ |
| LOD | 0.07 µg/spot | 0.08 µg/spot | 0. 78 µg $ml^{-1}$ |
| LOQ | 0.27 µg/spot | 0.28 µg/spot | 2.5 µg $ml^{-1}$ |
| Regression Parameters: | | | |
| - Slope ± SD ($S_b$) | 4.0523±2.054 E-02 | 3.965±5.786 E-03 | 0.100±7.220 E-5 |
| - Intercept ± SD ($S_a$) | 0.2052±1.018 E01 | 0.559±1.461 E02 | 0.241±4.080 E-6 |
| -SD of residual ($S_{xy}$) | 5.064 E-02 | 2.109 E-02 | 1.970 E-08 |
| Correlation coefficient | 0.9998 | 0.9994 | 0.9999 |
| Accuracy ± SD% | 101.0±1.04 | 100.3+1.51 | 99.7±0.59 |
| Precision * (RSD %) | | | |
| Intraday | 0.37-0.52 | 0.43-0.65 | 0.59-1.03 |
| Interday | 0.46-0.71 | 0.32-0.59 | 0.86-1.02 |

*n = 4

The detection limits (LOD) and quantitation limits (LOQ) were calculated on the basis of S.D. of the response and slope and were found to be 0.07-0.08 µg/spot and 0.27-0.28 µg/spot for the two drugs, respectively in the densitometric procedures and 0.78 and 2.5 µg $m1^{-1}$ for LZ in the $^{1}D$ procedure, Table (1).

The accuracy of the proposed procedures ranged between 98.8 and 101.0% for both drugs and their precision was evaluated by calculating the intraday RSD and found to be 0.37-1.03% while the interday RSD was 0.32-1.02% over a period of 3 weeks. The latter RSD proves the ruggedness of the proposed procedures.

The selectivity of the procedures was achieved by applying them to laboratory-prepared mixtures of intact LZ and PT-Na with their two degradates in different ratios, satisfactory results were obtained indicating that the proposed procedures were applicable for the selective determination of intact LZ in presence of up to 80% and 70% of its degradates by densitometry and $^{1}$D spectrophotometry, respectively. Also intact PT-Na can be determined in presence of up to 90% of its degradates by the densitometric procedure. Much higher results were obtained upon analyzing similar mixtures by the reported methods [5,7], Tables 2 and 3.

Table (2): Determination of Lansoprazole in Mixtures with its Degradates by the Proposed Procedures Compared with a Compendial Method[5].

| Densitometric procedure | | | ($^{1}$D) Spectrophotometric Procedure | | | Reported Method[5] | | |
|---|---|---|---|---|---|---|---|---|
| Intact (µg / spot) | Degraded* % | Recovery % of intact | Intact (µg / spot) | Degraded* % | Recovery % of intact | Intact (µg / ml) | Degraded* % | Recovery % of intact |
| 8.1 | 10 | 99.4 | 18 | 10 | 99.1 | 18 | 10 | 105.7 |
| 6.3 | 30 | 97.6 | 16 | 20 | 98.6 | 16 | 20 | 108.0 |
| 5.4 | 40 | 100.5 | 14 | 30 | 99.3 | 14 | 30 | 114.0 |
| 4.5 | 50 | 99.5 | 12 | 40 | 100.3 | 12 | 40 | 119.3 |
| 3.6 | 60 | 97.7 | 10 | 50 | 99.6 | 10 | 50 | 120.9 |
| 1.8 | 80 | 100.3 | 8 | 60 | 98.5 | 8 | 60 | 128.9 |
| | | | 6 | 70 | 100.3 | 6 | 70 | 132.0 |
| | | | 4 | 80 | 114.1** | 4 | 80 | 154.0 |
| | | | 2 | 90 | 124.5** | 2 | 90 | 206.8 |
| Mean±SD% | | 99.2±1.14 | | | 99.4±0.66 | | | |

* of total weight ** Rejected

The reported method is a direct UV measurement of lansoprazole in 0.1 N NaOH at 296 nm[5]

Table (3): Determination of Pantoprazole Sodium in Mixtures with its Degradates by the Proposed densitometric Procedure Compared with Compendial Method[7]

| Densitometric Procedure | | | Reported Method[7] | | |
|---|---|---|---|---|---|
| Intact (µg/spot) | Degraded* % | Recovery % of intact | Intact (µg/spot) | Degraded* % | Recovery % of intact |
| 4.0 | 20 | 100.4 | 45 | 10 | 123.8 |
| 3.5 | 30 | 98.1 | 40 | 20 | 124.3 |
| 2.5 | 50 | 99.4 | 35 | 30 | 124.9 |
| 1.5 | 70 | 99.1 | 30 | 40 | 126.84 |
| 1.0 | 80 | 99.7 | 25 | 50 | 140.6 |
| 0.5 | 90 | 98.3 | 20 | 60 | 151.3 |
| Mean ± SD % | | 99.2 ± 0.79 | | | |

* of total weight

The reported method is the measurement of the coloured product produced by the reaction of pantoprazole sodium with DDQ in acetonitrile at 457 nm. [7]

**Applicability**

Accuracies of the two procedures when applied to their formulations were almost the same as their accuracies when applied to pure drugs as evidenced by the recovery of standard added; 100.2±0.29 % and 100.0±0.94% for lanzor capsules by the densitometric and $^{1}$D procedures, respectively, also 99.8±0.82% for controloc tablets or 101.0±0.84% for pantoloc tablets by the densitometric procedure, Table (4). Statistical analysis of the results obtained by the two procedures, compared with the reported methods for LZ[5] and PT-Na[7] revealed no significant difference within a probability of 95%, Table (5). However, the proposed procedures are more sensitive and more selective than the reported methods which can not differentiate between the intact molecule and its degradates.

Table (4) Determination of LZ and PT-Na in pharmaceutical formulations by the proposed procedures.

| Formulation | Densitometric Procedure | | | | ($^1$D) Spectrophotometric Procedure | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean ±CV% | Standard Addition | | | Mean ±CV% | Standard Addition | | |
| | | Claimed (µg/spot) | Added (µg/spot) | Recovery% of added | | Claimed (µg/ml) | Added (µg/ml) | Recovery% of added |
| Lanzor capsules | 100.3 ± 1.31 | 2 | 4 | 100.0 | 99.4±0.36 | 3 | 22 | 99.6 |
| | | 2 | 5 | 100.0 | | 3 | 17 | 99.6 |
| | | 2 | 6 | 100.6 | | 3 | 12 | 99.3 |
| | | | | | | 3 | 7 | 101.7 |
| Mean±CV% | | | | 100.2±0.29 | | | | 100.0±0.94 |
| Controloc Tablets | 99.9±0.49 | 0.5 | 0.5 | 100.8 | | | | |
| | | 0.5 | 1.0 | 99.8 | | | | |
| | | 0.5 | 1.5 | 98.8 | | | | |
| Mean±CV% | | | | 99.8±0.82 | | | | |
| Pantoloc Tablets | | 1 | 1 | 99.8 | | | | |
| | | 1 | 2 | 101.8 | | | | |
| | | 1 | 3 | 101.3 | | | | |
| Mean±CV% | | | | 101.0±0.84 | | | | |

Table (5): Statistical Analysis of the Results Obtained by the Proposed Procedures and Compendial Methods[5,7]

| Parameter | Lanzor Capsules | | | Controloc Tablets | | Pantoloc Tablets | |
|---|---|---|---|---|---|---|---|
| | Densitometric procedure | ($^1$D) procedure | Reported Method[5] | Densitometric procedure | Reported Method[7] | Densitometric procedure | Reported Method[7] |
| Linearity | | 5-25 | | | | | |
| Range | 1-9 | µg ml$^{-1}$ | 3-25 | 0.5-4.0 | 10-60 | 0.5-4.0 | 10-60 |
| N | µg/spot | 5 | µg ml$^{-1}$ | µg / spot | µg ml$^{-1}$ | µg /spot | µg ml$^{-1}$ |
| Mean % | 4 | 99.4 | 5 | 4 | 6 | 4 | 6 |
| SD | 100.3 | 0.36 | 100.0 | 99.9 | 99.6 | 99.1 | 99.6 |
| Variance | 1.31 | 0.13 | 0.76 | 0.51 | 0.88 | 0.91 | 1.10 |
| t | 1.72 | 1.65 | 0.58 | 0.26 | 0.77 | 0.83 | 1.21 |
| F | 0.46 (2.36) | (2.31) | - | 0.61 (2.31) | - | 0.75 (2.31) | - |
| | 2.94 (9.12) | 4.46 (6.39) | - | 2.96 (9.01) | - | 1.45 (9.01) | - |

Figures in parantheses are the theoretical t and F values at P = 0.05

## Conclusion

LZ and PT-Na are highly sensitive drugs to heat, light, moisture, acidic and weakly basic substances which may destroy their activity. Thus, the establishment of the proposed densitometric and $^{1}$D- spectrophotometric procedures that selectively determine the pure drugs in their pharmaceutical formulations without interferences from excipients or degradation products provide a great pharmaceutical value. The densitometric procedure is more sensitive and more selective than the $^{1}$D procedure, while the latter one has the advantage of lower cost, rapidity and environmental protection. Nevertheless, the two procedures are suitable for quality control laboratories where simplicity, economy and time are essential.

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