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Stability-indicating methods for the determination of pipazethate HCl in the presence of its alkaline degradation product

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KEYWORDS

Pipazethate HCl;
Stability-indicating;
Ratio-spectra first derivative;
Densitometry;
HPLC technique

Abstract Three different accurate, sensitive and reproducible stability-indicating methods for the determination of pipazethate HCl in the presence of its alkaline degradation product are presented. The first method is based on ratio-spectra 1st derivative (RSD_1) spectrophotometry of the drug at 305 nm, over a concentration range of 10–70 μg mL^{-1} with mean percentage recovery of 99.69 ± 1.10. The second method utilises quantitative densitometric evaluation of thin-layer chromatography of pipazethate HCl in the presence of its alkaline degradation product, using methanol: ethyl acetate: ammonia (8:2:0.2, v/v/v) as a mobile phase. Chromatograms are scanned at 251 nm. This method analyses pipazethate HCl in a concentration range of 4–14 μg/spot with mean percentage recovery of 100.19 ± 0.77. The third method is an HPLC method for the simultaneous determination of pipazethate HCl in the presence of its alkaline degradation product. The mobile phase consists of methanol: ammonium sulphate (1%), pH = 5.7, (80:20, v/v). The standard curve of pipazethate HCl shows a good linearity over a concentration range of 5–200 μg mL^{-1} with mean percentage recovery of 100.67 ± 0.91. These methods were successfully applied to the determination of pipazethate HCl in bulk powder, laboratory-prepared mixtures containing different percentages of the degradation product and pharmaceutical dosage forms. The validity of results was assessed by applying standard addition technique. The results obtained were found to agree statistically with those obtained by a reported method, showing no significant difference with respect to accuracy and precision.

Introduction

Pipazethate HCl is 2-(2-piperidinoethoxy)ethyl 10H-pyrido [3,2-*b*] [1,4]benzothiadiazine-10-carboxylate hydrochloride [1], Fig. 1.

Pipazethate HCl is a non narcotic antitussive drug that acts by suppressing irritable and spasmodic cough by inhibiting the excitability of the cough centre and of peripheral neural receptors in the respiratory passage [2,3].

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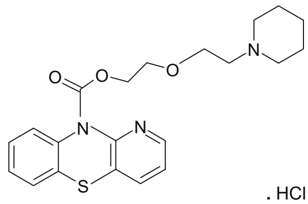


Figure 1 Chemical structure of pipazethate HCl, $C_{21}H_{25}N_3O_3S\cdot HCl$, M.Wt. = 436.

Several methods have been reported for the analysis of pipazethate HCl in both pure and pharmaceutical dosage forms; these include HPLC [4,5], qualitative TLC [6] and electrochemical methods [7,8].

HPLC has been performed by measuring peak area either at 230 nm using methanol: ammonium sulphate (1%) (85:15, v/v) as a mobile phase on ion exchange column [4], or at 276 nm using methanol: water (60:40, v/v) as a mobile phase on C18 column [5].

Spectrophotometric methods, measuring absorption at 251 nm in 0.1 N HCl solution [3,9] and colorimetric procedures with different dyes [10–12], have been described. Spectrophotometric methods based on the oxidation of the drug by Fe^{3+} in the presence of o-phenanthroline (o-phen) or bipyridyl (bipy); or reduction of Fe(III) by the drug in an acid medium and subsequent interaction of Fe(II) with ferricyanide to form Prussian blue, which exhibits an absorption maximum at 750 nm have also been reported [13].

Colorimetric methods, depending upon the reaction of cobalt(II)-thiocyanate or molybdenum(V)-thiocyanate ions with the cited drug to form stable ion-pair complexes, have been cited [14]. Another spectrophotometric method consists of extracting the formed ion-associates of the drug with chromotrope 2B or chromotrope 2R into chloroform and measuring the produced colours spectrophotometrically [15].

None of these methods is concerned with the analysis of pipazethate HCl in the presence of its alkaline degradation product, thus the aim of the present study was to develop simple and accurate stability-indicating methods for selective determination of pipazethate HCl in the presence of its alkaline degradation product with the application to pharmaceutical dosage forms that could be applied for drug quality control.

Experimental

Apparatus

All absorption spectra were recorded with a Shimadzu UV-1601 PC UV–Visible double beam spectrophotometer with 1 cm quartz cuvettes, Shimadzu Corporation, Kyoto-Japan.

Densitometer: dual wavelength Shimadzu flying CS-9000 with video display and high-speed, high-quality, parallel-head printer/plotter.

Hamilton micro-syringe, 25 µL or 100 µL, calibrated at 0.2 µL per unit.

Thin-layer chromatography (TLC) plates: pre-coated with Silica Gel GF_{254}, 20 × 20 cm, 0.25 mm thickness, (E. Merck, Darmstadt, Germany).

The HPLC system consisted of a Shimadzu LC-10 AD HPLC pump and a model SPD-10A Shimadzu UV–Visible detector. The analytical column was a Bondapak C18 (150 mm × 3.9 mm I.D., particle size 5 µm) from Waters, USA. The detector was operating at 230 nm and the sensitivity was set at 0.001 AUFS. The elution was isocratic with a flow rate of 0.5 ml min^{-1}.

The mobile phase was prepared by mixing methanol with 1% ammonium sulphate, 80:20 v/v, and the pH was adjusted to 5.7 with either dilute sulphuric acid or ammonia solution.

Materials

Samples

Pure sample. Pipazethate HCl was kindly supplied by Egyptian International Pharmaceutical Industries Co. (EIPICO), Cairo, Egypt. Its purity was found to be 100.60 ± 0.61 by a reported spectrophotometric method [3].

Pharmaceutical dosage forms. Selegon drops are claimed to contain 40 mg pipazethate HCl per 1 mL. Selegon 20 mg tablets and Selegon 10 mg suppositories (batch numbers 024891, 011047 and 032414, respectively) were purchased from the local market. All dosage forms were manufactured by Egyptian International Pharmaceutical Industries Co. (EIPICO), Cairo, Egypt.

Preparation of alkaline degraded sample. The alkaline degradation product was laboratory prepared by dissolving 100 mg of pure pipazethate HCl in the least amount of methanol, refluxed with 100 mL 2 M NaOH in a 500-mL flask for 5 h, as it was proved by TLC to be the time required for complete degradation of the drug. The formed precipitate was filtered, washed with distilled water (5 × 10 mL), transferred to a flat bottom dish and dried at 105 °C for 2 h. The residue left after drying was used as the alkaline degradation product of pipazethate HCl. Structure elucidation was conducted by IR and mass spectroscopy.

Chemicals

All chemicals and reagents were of pure spectroscopic analytical grade. 2 M NaOH, 0.1 N HCl, ammonium sulphate (96%), concentrated ammonia (specific gravity 0.91), methanol, dichloromethane and ethyl acetate were all obtained from El-Nasr Pharmaceutical Chemicals Co., Abu Zabaal, Cairo, Egypt.

De-ionised water and methyl alcohol (E. Merck, Darmstadt, Germany) were of HPLC grade.

Standard solutions

Stock solution of pipazethate HCl or its alkaline degradation product (100 µg mL^{-1}) in 0.1N HCl, for ratio-spectra 1st derivative (RSD_1), was prepared by dissolving 100 mg of pipazethate HCl powder or its alkaline degradation product in 0.1 N HCl in a 100-mL measuring flask. Ten millilitres of this solution were accurately transferred into a 100-mL measuring flask and the volume was completed with 0.1 N HCl.

Pipazethate HCl stock standard solution or its alkaline degradation product (1000 µg mL^{-1}) in methanol for spectrodensitometric and HPLC methods, were prepared by accurately weighing 100 mg of pipazethate HCl powder or its alkaline degradation product in a 100-mL measuring flask and dissolving in methanol.

Procedures

Ratio-spectra 1st derivative (RSD_1) spectrophotometric method
Construction of calibration curve. Accurately measured volumes of pipazethate HCl stock solution (100 μg mL^{-1}) were transferred into 10-mL measuring flasks, diluted to volume with 0.1 N HCl to get final concentrations 10–70 μg mL^{-1}. The absorption spectra of pipazethate HCl solutions were divided by the absorption spectra of the alkaline degradation product (20 μg mL^{-1}). The obtained ratio spectra were differentiated with respect to wavelength, and 1st derivative values at 305 nm were recorded. First derivative values were plotted versus the corresponding concentration and the regression equation was calculated. The experiment was repeated three times.

Assay of laboratory-prepared mixtures. Aliquots of pipazethate HCl stock solution (100 μg mL^{-1}) were accurately transferred into a series of 10-mL measuring flasks to get final concentrations of 90%, 80%, (…) 30% of pipazethate HCl. Aliquots of alkaline degradation product stock solution (100 μg mL^{-1}) were added to the same flasks to get final concentrations of 10%, 20%, (…) 70% of the alkaline degradation product. The volumes were completed with 0.1 N HCl and mixed thoroughly. The RSD_1 values were recorded at 305 nm. The concentration of pipazethate HCl was calculated from its regression equation. Each concentration was calculated from four experiments.

Spectrodensitometric method
Construction of calibration curve. Aliquot volumes (0.4, 0.6, … 1.4 mL) of pipazethate HCl standard stock solution (1000 μg mL^{-1}) were transferred into a series of 10 mL measuring flasks and diluted to volume with methanol. A sample of 100 μL was applied to a thin layer chromatographic plate (20 × 20) using a 25 μL Hamilton syringe. Spots were spaced 2 cm apart from each other and 2 cm from the bottom edge of the plate. The plate was developed in a chromatographic tank previously saturated for at least 1 h with the developing mobile phase; methanol: ethyl acetate: ammonia (8:2:0.2, v/v/v), by ascending mode. The plate was removed, dried in air and the spots were visualized under UV lamp at 254 nm and scanned at 251 nm. The calibration curve was plotted between the recorded area under the peak and the corresponding concentration, from which the regression equation was calculated. The calibration curve was made from the average of three experiments.

Assay of laboratory-prepared mixtures. Aliquots of pipazethate HCl stock solution (1000 μg mL^{-1}) were accurately transferred into a series of 10-mL measuring flasks to get final concentrations of 90%, 70%, (…) 10% of pipazethate HCl. Accurately measured volumes of alkaline degradation product stock solution (1000 μg mL^{-1}) were introduced to the same flasks to get final concentrations of 10%, 20%, (…) 90% of alkaline degradation product. Hundred microlitres of the prepared mixtures were applied to a silica gel plate and the procedure under 'Construction of calibration curve' was followed. The concentrations of pipazethate HCl were calculated from the corresponding regression equation. Four replicates for each experiment were conducted.

HPLC method
Construction of calibration curve. Accurately measured volumes of pipazethate HCl stock solution (1000 μg mL^{-1}) were transferred into 10-mL measuring flasks, diluted to the volume with the mobile phase to get the final concentration range of 5–200 μg mL^{-1}. Twenty microlitres of these solutions were injected into the HPLC system. The chromatograms were recorded and a calibration curve for pipazethate HCl was plotted and the corresponding regression equation was calculated. Triplicate experiments were performed.

Assay of laboratory-prepared mixtures. Aliquots of pipazethate HCl stock solution (1000 μg mL^{-1}) were accurately transferred into a series of 10-mL measuring flasks to get final concentrations of 90%, 70%, (…) 10% of pipazethate HCl. Portions of alkaline degradation product stock solution (1000 μg mL^{-1}) were introduced to the same flasks to get final concentrations of 10%, 30%, (…) 90% of alkaline degradation product, then the volume was completed to the mark with the mobile phase. The chromatographic conditions were adopted for each laboratory-prepared mixture and the concentration of pipazethate HCl was calculated from the regression equation. Each concentration was conducted from four experiments.

System suitability. Twenty microlitres of the solvent mixture and the working standard solutions were injected. The system suitability parameters, retention time, tailing factor, theoretical plate count (*N*), height of theoretical plate (HETP), separation of pipazethate HCl peak and its degradation product peak (resolution) and column capacity were calculated.

Application to pharmaceutical dosage forms
Selegon drops. Accurately measured 2.5 mL selegon drops (1 mL = 40 mg pipazethate HCl), were transferred into a 100-mL measuring flask and the volume was completed to the mark with 0.1 N HCl, for RSD1 method, or with methanol, for densitometric and HPLC methods (1000 μg mL^{-1}). Ten millilitres of this drop solution (1000 μg mL^{-1}) was transferred into a 100 mL measuring flask and diluted to the mark with 0.1 N HCl to get a final concentration of 100 μg mL^{-1}, then the procedures under 'Construction of calibration curves' for each method were followed. Four replicates for each experiment were done.

Selegon tablets. Twenty selegon tablets were weighed and powdered. A portion of the powder equivalent to 100 mg pipazethate HCl was accurately weighed into a 100 mL beaker, stirred with 0.1 N HCl, for RSD1 method, or with methanol, for densitometric and HPLC methods (4 × 20 mL) and filtered into a 100-mL measuring flask. The volume was completed with the same solvent (1000 μg mL^{-1}). Ten millilitres of this tablet stock solution (1000 μg mL^{-1}) was transferred into a 100 mL measuring flask and diluted to the mark with 0.1 N HCl to get a final concentration of 100 μg mL^{-1}, then the procedures under 'Construction of calibration curves' for each method were followed. Each concentration was done from four experiments.

Selegon suppositories. Twenty selegon suppositories were melted and mixed well. A quantity containing 100 mg of pipazethate HCl was weighed and accurately transferred into a 100 mL beaker, extracted by shaking with 0.1 N HCl, for RSD1 method, or with methanol, for densitometric and HPLC methods (4×20 ml) and decanted through filter paper into a 100-mL measuring flask. The volume was completed with the same solvent (1000 μg mL^{-1}). 10 mL of this suppository stock solution (1000 μg mL^{-1}) was transferred into 100 mL measuring flask and diluted to the mark with 0.1 N HCl to get a final concentration of 100 μg mL^{-1}, then the procedures under 'Construction of calibration curves' for each method were performed. Four replicates for each experiment were done.

Results and discussion

Many pharmaceutical compounds undergo degradation during storage or even during the different processes of their manufacture. Several chemical or physical factors can lead to the degradation of drugs [16]. Hydrolysis and oxidation are the most famous chemical degradation routes of drugs [17,18]. The main classes of drugs that are subject to degradation are esters, amides and lactams. Ester hydrolysis is frequently base catalysed, which makes the reaction rapid and irreversible [17,19].

Pipazethate HCl has an ester linkage, so trials were conducted for its degradation in either an acidic or basic medium. It was found that the drug was liable to degradation upon refluxing in a strong basic medium to give two degradates. One is the alcohol derived from the hydrolysis of the ester group of the drug. This alcohol has no absorption at 251 nm, as it has no chromophoric group, thus it does not interfere with the determination of the intact drug. The other is the free base which remains after decarboxylation under the conditions of the reaction demonstrated in the following scheme (Scheme 1).

In this work, alkali-hydrolysed pipazethate HCl degradation product was prepared, separated and its structure identified by mass spectroscopy. It shows the parent peak at 201 m/z while the peak of pipazethate HCl is at 398 m/z. This indicates that the ester group suffered cleavage by 2 M NaOH leading to the formation of the corresponding base (after decarboxylation). This was further confirmed by IR spectroscopy. IR spectroscopy of the degradation product showed the disappearance of the carbonyl band at 1750 cm^{-1}.

The present work was conducted for the selective determination of pipazethate HCl in the presence of its alkali-hydrolysed degradation product with the application to pharmaceutical dosage forms.

Ratio-spectra 1st derivative (RSD_1) spectrophotometric method

Ratio-spectra 1st derivative spectrophotometry (RSD_1) is an analytical technique of good utility which offers background correction and better selectivity than normal spectrophotometry for resolving binary mixtures and some ternary mixtures [20].

The zero-order absorption spectra of pipazethate HCl and its degradation product showed severe overlap over the entire spectrum of the intact drug, Fig. 2. Therefore, the use of direct absorbance measurements for assaying pipazethate HCl in the presence of its degradation product was not possible.

The 1st, 2nd, 3rd and 4th order absorption spectra of pipazethate HCl in the presence of its alkaline degradation product showed severe spectral overlap with no zero crossing points. Therefore ratio-spectra 1st derivative (RSD_1) method was suggested to solve this problem.

The theory of derivative ratio spectrophotometry, which is based on the use of first (or second) derivatives of the ratio spectra of the mixture and divided (amplitudes at each wavelength) by the absorption spectrum of a standard solution of one of the components, has been applied extensively to the simultaneous determination of substances with overlapping spectra as an economic alternative to HPLC methods [21], and to solve the problem of overlapping absorption spectra of pipazethate HCl and its alkaline degradation product. In the present investigation, the careful choice of the divisor and the working wavelength were of great importance as it affected both sensitivity and selectivity; accordingly, different concentrations of the degradation product (10, 20, 30 and 60 μg mL^{-1}) were tried as divisors. It was found that

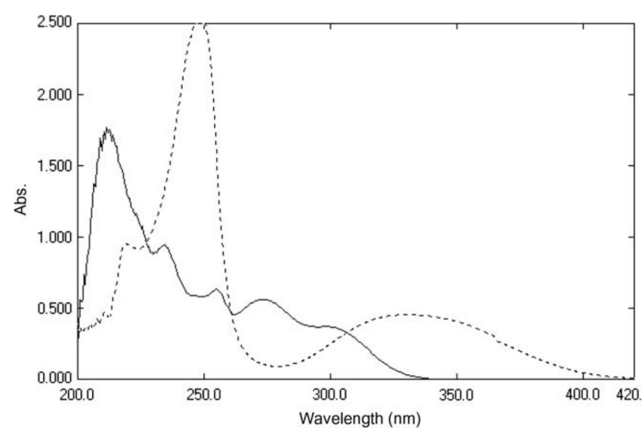
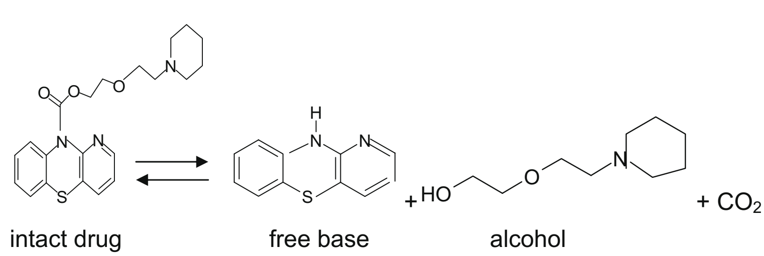


Figure 2 Absorption spectra of 50 μg mL^{-1} pipazethate HCl (–) and 20 μg mL^{-1} of its alkaline degradation product (...).



Scheme 1

20 μg mL^{-1} was the best, as it produced minimum noise and gave better results in agreement with selectivity.

Pipazethate HCl was assayed by dividing the absorption spectra of different concentrations in the range of 10–70 μg mL^{-1} by the absorption spectra of 20 μg mL^{-1} alkaline degradation product, Fig. 3. The obtained ratio spectra were differentiated with respect to wavelength, Fig. 4. The RSD_1 values showed good linearity and accuracy. The regression equation was computed to be:

$$Y = 0.211X - 0.0021 \quad r = 0.9998,$$

where Y is the RSD_1 value at 305 nm, X is the concentration in μg mL^{-1} and r is the correlation coefficient.

Determination of pipazethate HCl in the presence of its alkaline degradation product could also be performed by RSD_1 at 273 nm. This wavelength showed good linearity and accuracy, but less than at 305 nm.

Results obtained in Table 1 show that the proposed method is valid and applicable for simultaneous determination of pipazethate HCl in the presence of up to 70% of the alkaline degradation product in different laboratory-prepared mixtures with mean percentage recovery 99.38 ± 0.82.

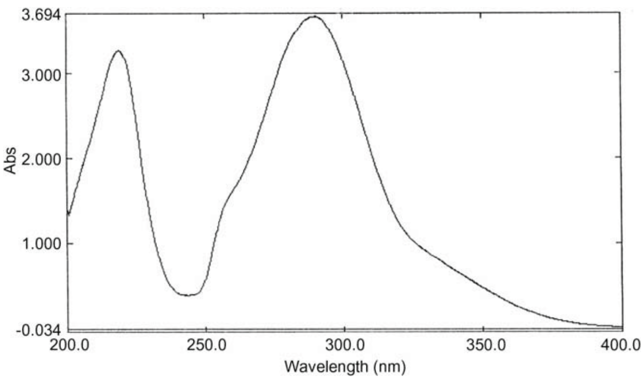


Figure 3 Ratio spectrum of 50 μg mL^{-1} pipazethate HCl using 20 μg mL^{-1} of its alkaline degradation product as a divisor.

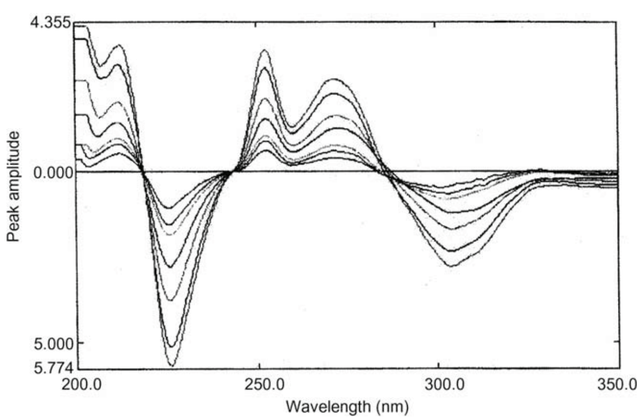


Figure 4 First derivative ratio spectra of pipazethate HCl (10–70 μg mL^{-1}) using 20 μg mL^{-1} of its alkaline degradation product as a divisor.

Table 1 Determination of pipazethate HCl in laboratory-prepared mixtures by the proposed RSD_1 method.

Mixture no.	Alkaline degradate added%	Pipazethate HCl		
		Taken (μg mL^{-1})	Found[a] (μg mL^{-1})	Recovery (%)
1	10.00	63.00	62.47	99.16
2	20.00	56.00	55.98	99.96
3	25.00	52.50	52.09	99.22
4	30.00	49.00	48.11	98.18
5	40.00	42.00	42.04	100.10
6	50.00	35.00	34.60	98.86
7	60.00	28.00	28.20	100.70
8	70.00	21.00	20.76	98.87
Mean ± SD				99.38 ± 0.82

[a] Average of four determinations.

Spectrodensitometric method

TLC densitometry overcomes the problem of overlapping absorption spectra of a mixture of drugs by separating these components on TLC plates and determining each ingredient by scanning the corresponding chromatogram. The TLC–UV densitometric method has the advantage of simultaneously determining the active ingredients in multi-component dosage forms [22].

The proposed procedure is based on the difference in R_f values of pipazethate HCl (R_f = 0.28) and its alkaline degradation product (R_f = 0.51). Various developing systems were tried, but complete separation was achieved using methanol: ethyl acetate: ammonia (8:2:0.2, v/v/v).

The separated spots from different concentrations of the drug were scanned at 251 nm. A linear relation was obtained between peak area and concentration in the range of 4–14 μg/spot, from which the linear regression equation was found to be:

$$Y = 0.1053X + 0.2469 \quad r = 0.9995,$$

where Y is the area under the peak, X is the concentration in μg/spot and r is the correlation coefficient.

The results obtained during analysis of laboratory-prepared mixtures, Table 2, show that the method is valid for the

Table 2 Determination of pipazethate HCl in laboratory-prepared mixtures by the suggested TLC densitometric method.

Mixture no.	Alkaline degradate added (%)	Pipazethate HCl		
		Taken (μg mL^{-1})	Found[a] (μg mL^{-1})	Recovery (%)
1	10.00	12.60	12.59	99.99
2	20.00	11.20	11.28	100.71
3	40.00	8.40	8.36	99.52
4	60.00	5.60	5.62	100.36
5	70.00	4.20	4.22	100.48
Mean ± SD				100.21 ± 0.47

[a] Average of four determinations.

determination of intact pipazethate HCl in the presence of its alkaline degradation product up to 90% alkaline degradation product in different laboratory-prepared mixtures with mean percentage recovery of 100.21 ± 0.47.

HPLC method

A simple HPLC method was adopted for the simultaneous determination of pipazethate HCl in the presence of its alkaline degradation product without pervious separation.

Different mobile systems were tried, methanol: acetate buffer with different ratios and pH or with ammonium sulphate, for the chromatographic separation of the drug from its alkaline degradation product. The best resolution was achieved when using a mobile phase consisting of methanol: 1% ammonium sulphate (pH = 5.73) (80:20, v/v) using UV detection at 230 nm, which gave a better sensitivity for both drug and its alkaline degradation product.

A linear relation was obtained between peak area and the concentration of pipazethate HCl in the range of 5–200 μg mL^{-1}. The linear regression equation was found to be:

$$Y = 0.10057X + 0.2723 \quad r = 0.9999,$$

where Y is the area under the peak, X is the concentration in μg mL^{-1} and r is the correlation coefficient.

Results obtained by applying the HPLC procedure showed that pipazethate HCl can be simultaneously analysed in the presence of its alkaline degradation product in the laboratory-prepared mixtures, Table 5. The method is valid for the determination of intact pipazethate HCl in the presence of up to 90% alkaline degradation product, which was considered as the maximum expected degradation product to be available in a sample product in different laboratory-prepared mixtures with mean percentage recovery of 100.95 ± 0.33; Table 3.

The proposed methods have been applied to assay pipazethate HCl in selegon drops, tablets and suppositories. The validity of the suggested procedures was further assessed by applying the standard addition method, Table 4.

System suitability tests, which are used to ensure system performance before or during the analysis of drugs, were performed. The obtained values of pipazethate HCl and its alka-

Table 3 Determination of pipazethate HCl in laboratory-prepared mixtures by the elaborated HPLC method.

Mixture no.	Alkaline degradate added (%)	Pipazethate HCl		
		Taken (μg mL^{-1})	Found[a] (μg mL^{-1})	Recovery (%)
1	10.00	180.00	181.01	100.56
2	30.00	140.00	141.01	100.72
3	50.00	100.00	100.97	100.97
4	70.00	60.00	60.66	101.10
5	90.00	20.00	20.28	101.40
Mean ± SD				100.95 ± 0.33

[a] Average of four determinations.

Table 4 Application of standard addition technique for the analysis of pipazethate HCl in its pharmaceutical dosage forms by the proposed RSD_1, TLC densitometric and HPLC methods.

Product	Method	Found[a] (%)	Recovery (%)
Selegon drops B. N. 024891	RSD_1	99.39 ± 0.92	100.16 ± 0.75
Selegon tablets B. N. 011047		99.36 ± 0.59	99.60 ± 1.17
Selegon suppositories B. N. 032414		99.92 ± 1.01	100.64 ± 0.85
Selegon drops B. N. 024851	TLC	100.30 ± 0.23	100.23 ± 0.23
Selegon tablets B. N. 025149		100.29 ± 0.37	100.00 ± 0.59
Selegon suppositories B. N. 042720		99.08 ± 0.97	99.67 ± 0.32
Selegon drops B.N. 032368	HPLC	101.54 ± 0.62	101.42 ± 0.54
Selegon tablets B.N. 011047		99.69 ± 0.84	100.98 ± 0.72
Selegon suppositories B.N. 0321414		99.39 ± 1.01	99.38 ± 0.46

[a] Average of four determinations.

Table 5 System suitability parameters of the elaborated HPLC method for the analysis of pipazethate HCl in the presence of its alkaline degradation product.

Parameter	Obtained value	Reference value [23]
Resolution (R)	1.04	$R > 0.8$
T (tailing factor)	1	$T = 1$ for a typical symmetric peak
Relative retention time	1.94	>1
K (column capacity)	Pipazethate HCl (1.27) alkaline degradate (3.42)	1–10 acceptable
N (column efficiency)	Pipazethate HCl (483.2) alkaline degradate (1393.4)	Increases with efficiency of the separation
HETP (height equivalent to theoretical plates)	Pipazethate HCl (.0668) alkaline degradate (.0099)	The smaller the value. The higher the column efficiency

line degradation product were agreed with the stated reference values [23], Table 5.

A statistical comparison of the results obtained by the proposed methods and a reported method [3] for pure drug is shown in Table 6. The values of the calculated t and F were less than the corresponding tabulated ones, which revealed that there was no significant differences with respect to accuracy and precision between the proposed methods and the reported procedure.

Assay validation was done by repeating the procedures three times on three different days (inter-day) and three times on different times intervals within the same day (intraday) for the analysis of different concentrations of pipazethate HCl, Table 7. The results show that the methods were accurate, precise and specific.

The robustness of the methods and their ability to remain unaffected by small changes in parameters were tested. Variation of pH of the mobile phase by ±0.2 and its organic solvent concentration by 4% did not have a significant effect on chromatographic resolution of the HPLC method. Variation of the concentration of HCl by ±0.02 M did not have significant effect on spectrophotometric methods.

Table 6 Statistical analysis of the results obtained by applying the proposed methods and a reported spectrophotometric method for the analysis of pure pipazethate HCl.

Values	RSD_1 method	TLC densitometric method	HPLC method	Reported method [3]
Mean	99.69	100.19	100.67	100.60
±SD	1.10	0.77	0.91	0.61
n	7	8	7	7
Variance	1.21	0.59	0.83	0.37
t	1.912 (2.179)*	1.133 (2.16)*	0.169 (2.179)*	–
F	3.27 (4.28)*	1.59 (4.21)*	2.24 (4.28)*	–

Table 7 Validation of the results obtained by applying the suggested methods for the determination of pipazethate HCl.

Parameters	RSD_1 method	TLC densitometric method	HPLC method
Range	10–70 μg mL^{-1}	4–14 μg/spot	5–200 μg mL^{-1}
Slope	0.211	0.15053	0.10057
Intercept	−0.0027	0.2469	0.2723
Accuracy	99.69 ± 1.1	100.19 ± 0.77	100.67 ± 0.91
Specificity	99.38 ± 0.82	100.05 ± 0.56	100.95 ± 0.33
Variance	1.21	0.59	0.83
Correlation coefficient (r)	0.9998	0.9995	0.9999
RSD (%)	1.10	0.77	0.90
Repeatability [a]*	99.72 ± 0.38	100.25 ± 1.22	100.75 ± 0.98
Intermediate precision [b]*	99.94 ± 0.49	101.51 ± 1.96	100.93 ± 1.30
LOD[c]*	7.00	2.00	1.00
LOQ[c]*	10.00	3.00	5.00

[a] The intraday mean value ± standard deviations of samples of pipazethate HCl (20, 40, 60 μg mL^{-1}) for RSD_1 method, (4, 6, 8 μg/spot) for TLC densitometric method and (20, 50, 100 μg mL^{-1}) for HPLC method.

[b] The inter-day mean value ± standard deviations of samples of pipazethate HCl (20, 40, 60 μg mL^{-1}) for RSD_1 method, (4, 6, 8 μg/spot) for TLC densitometric method and (20, 50, 100 μg mL^{-1}) for HPLC method.

[c] LOD and LOQ were done practically.

Conclusion

Three methods, RSD_1, TLC and HPLC were developed for the determination of pipazethate HCl in the presence of its alkaline degradation product. The methods provide simple, accurate, rapid and reproducible quantitative analysis of pipazethate HCl in bulk powder, laboratory-prepared mixtures and dosage forms.

The RSD_1 method has the advantages of being more economical, rapid and environmentally secure than the other methods. The TLC method was found to be more sensitive than the RSD_1 method. The proposed HPLC method gives a good resolution between pipazethate HCl and its alkaline degradation products within a short time and a dynamic range. These methods can be used as stability-indicating procedures in quality control laboratories where economy and time are essential.

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