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THREE REVERSED PHASE LIQUID CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF SOME ANTICHOLENERGIC DRUGS IN THE PRESENCE OF THEIR DEGRADATION PRODUCTS AND / OR IN MIXTURE WITH OTHER DRUGS

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تم فى هذا البحث عمل ثلاث طرق كروماتوجرافيا السائل ذات الأداء العالى لتقدير بعض الأدوية المستخدمة فى علاج اضطرابات الجهاز الهضمى فى وجود نواتج تحللها القلوية وفى مخاليطها مـــع أدوية أخرى.

فى الطريقة الأولى تم تقدير البيبكسولان هيدروكلوريد فى وجود نواتج تحللة القلوية باستخدام السائل المتحرك المكون من الأسيتونيتريل: ٠٫٠٢م محلول الفوسفات الذى تم ضبط الأس الهيدروجينى له عند ٧ر٥ (٣٠:٧٠) وقد تم قياس المواد المنفصله عند طول الموجه ٢١٥ ن.م. وفى الطريقة الثانية تم تقدير كلا من الدروفينيين هيدروكلوريد والبروبيفينازون فى وجود ناتج التحلل القلوى للدروفينين وذلك باستخدام السائل المتحرك الأسيتونيتريل: ٠ر٠٥م محلول الفوسفات الذى تم ضبط الأس الهيدروجينى له عند ٣ر٥ (٦٠:٤٠) وقد تم قياس المواد المنفصله عند طول الموجه ٢١٥ ن.م. أما الطريقة الثالثة فتم فيها تقدير الايزوبروباميد أيوديد والترای فلوبيرازين هيدروكلوريد باستخدام السائل المتحرك المكون من الأسيتونيتريل: ٠ر٠٥م محلول الفوسفات المحتوى على ١% ترای ايثيل امين الذى تم ضبط الأس الهيدروجينى له عند ٣ر٥(٥٠:٥٠) وقد تم قياس المواد المنفصله عند طول الموجه ٢١٠ ن.م.

Three RP-LC methods have been developed for the quantitative determination of some anticholinergic drugs in the presence of their degradation products and/or in mixture with other drugs. In method (I) pipoxolan HCl is estimated in the presence of its alkaline-induced degradation products, 0.02 M phosphate buffer pH 7.5: acetonitrile (30:70 v/v) was used as a mobile phase with UV detection at 215 nm. Drofenine HCl was used as an internal standard. Method (II) describes the simultaneous determination of drofenine HCl and propyphenazone in the presence of drofenine HCl alkaline-induced degradation product. This method used 0.05 M phosphate buffer pH 3.5: acetonitrile (60:40 v/v) as a mobile phase with UV detection at 215 nm. In method (III) the simultaneous determination of isopropamide iodide and triflouperazine HCl is presented. In this method 0.05 M phosphate buffer (containing 0.1% triethylamine) pH 3.5: acetonitrile (50:50 v/v) was used as a mobile phase with UV detection at 210 nm. Pipoxolan HCl was used as an internal standard in the determination of the two binary mixtures.

INTRODUCTION

Pipoxolan hydrochloride, 5, 5-diphenyl-2-(2-piperidinoethyl)-1, 3-dioxolan-4-one, is a smooth muscle relaxant[1] used in the treatment of spasms and colic due to irritable bowel syndrome. In 2010, it was reported that pipoxolan HCl inhibits the proliferation of HL-60 human leukemia cancer cell[2]. Its dioxolan moiety was thought to induce apoptosis in cancer cells[3].

Drofenine hydrochloride, 2-(diethyl-amino)ethyl α-phenylcyclohexaneacetate

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hydrochloride, is an antimuscarinic drug used in the treatment of visceral spasms[1].

Isopropamide iodide, (3-carbamoyl-3,3-diphenylpropyl) di-isopropylmethyl ammonium iodide, is a quaternary ammonium anti-muscarinic with peripheral effects similar to those of atropine. It is used as an adjunct in the treatment of peptic ulcer disease, in the relief of gastrointestinal and urinary-tract disorders associated with smooth muscle spasm[1].

The combination of isopropamide iodide with triflouperazine HCl provides an optimal and rapid relief in painful conditions associated with visceral spasm.

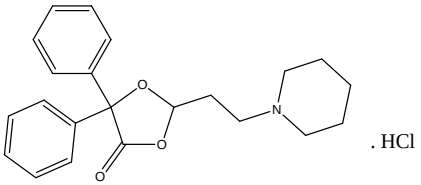
Propyphenazone, 4-isopropyl-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one, is a pyrazolone derivative related to phenazone has analgesic and antipyretic properties[1]

Triflouperazine hydrochloride, 10-[3-(4-methylpiperazin-1-yl)propyl]-2-trifluoro-methylphenothiazine dihydrochloride, is a phenothiazine antipsychotic drug causes sedation. It is used for the treatment of schizophrenia and other psychoses and for the control of nausea and vomiting[1].

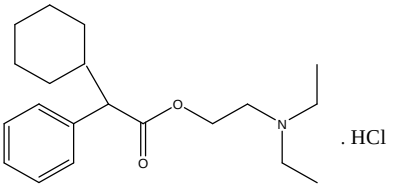
There is no literature was reported for the determination of pipoxolan HCl.

Several methods have been reported for the determination of drofenine HCl with allobarbital and aminophenazone by gas chromatography[4] and by LC methods[5-7].

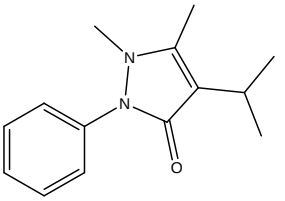
Several methods have been reported for the determination of isopropamide iodide with triflouperazine HCl by spectrophotometric methods[8-10] and with other drugs by LC methods[11-13].



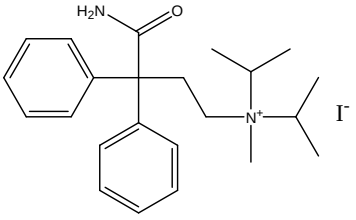
Pipoxolan HCl



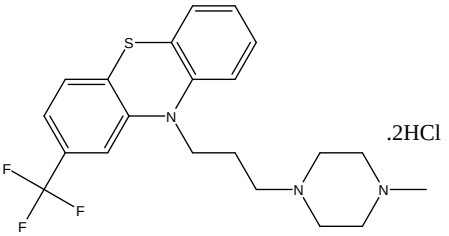
Drofenine HCl



Propyphenazone



Isopropamide iodide



Triflouperazine HCl

Chemical structure of pipoxolan HCl, drofenine HCl, propyphenazone, isopropamide iodide and triflouperazine HCl.

EXPERIMENTAL

Materials

Pipoxolan HCl working standard was kindly supplied by Amoun pharmaceutical Co., Cairo, Egypt. Its purity was found to be 100.33 ± 0.587 (n=6) according to the manufacturer's method[14]. Drofenine HCl and propyphenazone were kindly supplied by Pharco Co., Alex, Egypt. Their purities were found to be 99.84 ± 0.584 and 99.51 ± 0.795 (n=6) according to the manufacturer's method[15] for drofenine HCl and European pharmacopoeia (2008)[16] for propyphenazone respectively. Isopropamide iodide and triflouperazine HCl were kindly supplied by Kahira pharmaceutical and chemical Industrial Co., Cairo, Egypt. Their purities were found to be 100.04 ± 0.788 and 99.50 ± 0.584 (n=6) according to USP 32[17] for isopropamide iodide and European pharmacopoeia (2008)[16] for triflouperazine HCl.

Dosage forms

Rowapraxin tablets (Amoun pharmaceutical Co., Cairo, Egypt). Each tablet contains 10 mg pipoxolan HCl.

Spasmo-cibalgin tablets (Novartis pharma AG Basle, Zwitzerland). Each tablet contains 220 mg propyphenazone and 20 mg drofenine HCl.

Stellamide tablets (Kahira pharmaceutical and chemical Industrial Co., Cairo, Egypt). Each tablet contains 1 mg triflouperazine HCl and 5 mg isopropamide iodide.

Apparatus

a- Balance (scaltec, Germany)

b- Microprocessor pH meter – pH meter 211 (Hanna, Portugal).

c- Filter – disposable Nylon membrane filters 0.45 µm pore size (Whatman, International Ltd, Maidtone, England).

d- The LC system – consisting of Agilent 1100 series, interface equipped with an Agilent degasser JP3060993 (Japan), an Agilent quaternary pump DE62962767 (Germany), an Agilent manual injector DE60558338 equipped with (100 µl) injector loop, an Agilent column thermostat DE63065412 (Germany) and an Agilent UV-visible detector (Germany).

Agilent syringe, LC 250 µl (USA).

Hypersil BDS-C8 column - 4 x 250 mm, 5 µm, SN USUE000254 (USA) was used in method (I) and (II).

Hypersil thermo-C8 column - 4.6 x 250 mm, 5 µm, SN 0471929M (USA) was used in method (III).

Chemicals and reagents

All chemicals and solvents were analytical grade otherwise specified.

a- Chemicals - Sodium hydroxide and potassium dihydrogen orthophosphate (pure lab. EL-Nasr pharmaceutical Co., Egypt). Hydrocholric acid 37% (Riedel-de Haen, Germany). Orthophosphoric acid analar (BDH, England). Triethylamine (s d fine-chem limited, Mumbai)

b- Solvents - Acetonitrile (LC grade s d fine-chem limited, Mumbai)

Distilled water

c- Diluting solution – Acetonitrile : water (50:50 v/v)

d- Mobile phases- All mobile phases were filtered through 0.45 µm membrane filter and degassed for 30 min in an ultrasonic bath prior to its use.

In method (I): 0.02 M phosphate buffer pH 7.5 (prepared by dissolving 2.72 gm potassium dihydrogen orthophosphate in 900 ml distilled water adjusted to pH 7.5 with 10% sodium hydroxide to a pH 7.5±0.1 and diluted with distilled water to 1000 ml): acetonitrile (30:70 v/v).

In method (II): 0.05 M phosphate buffer pH 3.5 (prepared by dissolving 6.8 gm potassium dihydrogen orthophosphate in 900 ml distilled water, adjusted to pH 3.5±0.1 with orthophosphoric acid and diluted with distilled water to 1000 ml): acetonitrile (60:40 v/v).

In method (III): 0.05 M phosphate buffer pH 3.5 (prepared by dissolving 6.8 gm potassium dihydrogen orthophosphate in 900 ml distilled water containing 1ml triethylamine and adjusted to pH 3.5± with orthophosphoric acid then diluted with distilled water to 1000 ml): acetonitrile (50:50 v/v).

Preparation of sample

Standard solutions

Pipoxolan HCl stock solution (0.1 mg ml^{-1}) was prepared by dissolving accurately weighed 10 mg of into 100 ml volumetric flask, and completing to the volume with the diluting solution.

Drofenine HCl stock solution (0.2 mg/ml) and propyphenazone (0.1 mg/ml) were prepared by introducing accurately weighed 20 mg of and 10 mg of into two separate 100 ml volumetric flasks, dissolving and completing to the volume with the diluting solution.

Isopropamide iodide and triflouperazine HCl stock solutions (0.1 mg/ml) each were prepared by dissolving accurately weighed 10 mg of and 10 mg of into two separate 100 ml volumetric flasks, and completing to the volume with the diluting solution.

Degradation product stock solutions

An accurate weight (50 mg) of each of pipoxolan HCl and drofenine HCl was refluxed separately with 25 ml 1N NaOH for 4 hr then neutralize with 1N HCl and completed to 100 ml with water. Accurately measured aliquots of these solutions equivalent to 10 mg of pipoxolan HCl and 20 mg of drofenine HCl were introduced separately into two 100 ml volumetric flasks and completed to the volume with the diluting solution.

Calibration

For pipoxolan HCl (method I)

Accurately measured aliquots equivalent to (100-600 µg) of pipoxolan HCl from its stock solution were transferred into a series of 10 ml volumetric flasks. An aliquot equivalent to (50 µg) of drofenine HCl (internal standard) was added to each volumetric flask and the volume was completed with the diluting solvent. A volume 100 µl of each dilution was injected in triplicate; the chromatograms were recorded using 0.02M phosphate buffer pH 7.5: acetonitrile (30:70 v/v) as the mobile phase. The elution was carried out at ambient temperature using UV detection at 215 nm and a flow rate 1.5 ml/min.

For drofenine HCl and propyphenazone (method II)

Accurately measured aliquots equivalent to (200-1400 µg) of drofenine HCl and (100-700 µg) of propyphenazone from their stock solutions were transferred separately into two series of 10 ml volumetric flasks. An aliquot equivalent to (50 µg) of pipoxolan HCl (internal standard) was added to each volumetric flask and the volume was completed with the diluting solvent. A volume 100 µl of each dilution was injected in triplicate; the chromatograms were recorded using 0.05M phosphate buffer pH 3.5: acetonitrile (60:40 v/v) as the mobile phase. The elution was carried out at ambient temperature using UV detection at 215 nm and a flow rate 1.5 ml/min.

For isopropamide iodide and triflouperazine HCl (method III)

Accurately measured aliquots equivalent to (100-600 µg) of isopropamide iodide and (100-600 µg) of triflouperazine HCl from their stock solutions were transferred separately into two series of 10 ml volumetric flasks. An aliquot equivalent to (50 µg) of pipoxolan HCl (internal standard) was added to each volumetric flask and the volume was completed with the diluting solvent. A volume 100 µl of each dilution was injected in triplicate, the chromatograms were recorded using 0.05 M phosphate buffer pH 3.5 (containing 1ml/l triethylamine): acetonitrile (50:50 v/v) as the mobile phase. The elution was carried out at ambient temperature using UV detection at 210 nm and a flow rate 1.5 ml/min.

The ratios (R) of the recorded area under the peak (AUP) of each drug to that of the internal standard were plotted versus the corresponding concentrations in µg/ml to obtain the calibration curve of each drug and the corresponding regression equations were computed.

Assay of laboratory prepared mixtures

For pipoxolan HCl

Aliquots from pipoxolan HCl and its alkaline-induced degradation products (1&2) stock solutions equivalent to pipoxolan HCl (0.20 – 0.55 mg) and degradation products (1&2) (0.05 – 0.40 mg) were transferred into one series of 10 ml volumetric flasks. An aliquot equivalent to (50 µg) of drofenine HCl (internal standard) was added to each flask and the volume was completed with the diluting solution.

For drofenine HCl

Aliquots from drofenine HCl and its alkaline-induced degradation product (3) stock solutions equivalent to drofenine HCl (0.55 – 1.25 mg) and degradation product (3) (0.15 – 0.85 mg) were transferred into one series of 10 ml volumetric flasks. An aliquot equivalent to (50 µg) of pipoxolan HCl (internal standard) was added to each flask and the volume was completed with the diluting solution.

The chromatographic conditions were applied for each laboratory prepared mixture and the concentrations of each drug in these mixtures were calculated by substituting the regression equation for each drug.

Application to pharmaceutical preparations

Twenty tablets of each of Rowapraxine tablets, Spasmocebalgin tablets and Stellamide tablets were weighed, ground and an accurate weight of the powdered tablets equivalent to (10 mg) pipoxolan HCl in case of Rowapraxine tablets, (20 mg) drofenine HCl in case of Spasmocebalgin tablets and (10 mg) isopropamide iodide in case of Stellamide tablets were introduced separately into 100 ml volumetric flask, 80 ml diluting solution was added and the solution was sonicated for 30 min. The volume was completed with the diluting solution, (0.1 mg/ml) pipoxolan HCl in case of Rowapraxine tablets, (0.2 mg/ml) drofenine HCl in case of Spasmocebalgin tablets and (0.1 mg/ml) isopropamide iodide in case of Stellamide tablets the solutions were filtered and the first 10 ml of the filtrate was rejected.

Each pharmaceutical preparation was analyzed according to instrumental parameter as under linearity.

RESULTS AND DISCUSSION

Pipoxolan HCl containing a dioxolan moiety which is important for its activity[3] so it was necessary to develop simple stability indicating assay method for the determination of pipoxolan HCl in presence of its alkaline-induced degradation products. However, by reviewing the literature concerning the determination of this drug, it was found that no literature was reported for the determination of pipoxolan HCl by LC either alone or with its degradation products. Several mobile phases with different ratios for their components were tried such as:

- Water pH 4.5: methanol (25–50 : 75–50 v/v).
- Water pH 4.5: acetonitrile (25–50 : 75–50 v/v).
- Phosphate buffer pH 4.5 : acetonitrile (30–70 : 70–30 v/v).
- Water : methanol : acetonitrile (70:15:15, 50:25:25, 20:40:40 v/v/v)
- 0.02M phosphate buffer pH7.5: acetonitrile (30-75 : 70-30 v/v).

A satisfactory separation was obtained with the mobile phase 0.02 M phosphate buffer pH 7.5 : acetonitrile (30:70 v/v) as it showed good separation of pipoxolan HCl and degradation products (1&2) at retention time for pipoxolan HCl 4.994 min and for degradation products (1&2) 1.815 min and 2.400 min, respectively. Drofenine HCl was chosen as internal standard as it eluted at reasonable retention time 7.046 min and showed good separation from mixture components (Fig. 1). The formation of two degradation products of much higher polarity than pipoxolan HCl as shown by their retention time [degradation product (1) 1.815, degradation product (2) 2.400 and pipoxolan HCl 4.994 min] could be attributed to the cleavage of the lactone group giving a product with a COOH & OH groups [degradation product (1) 1.815 min].

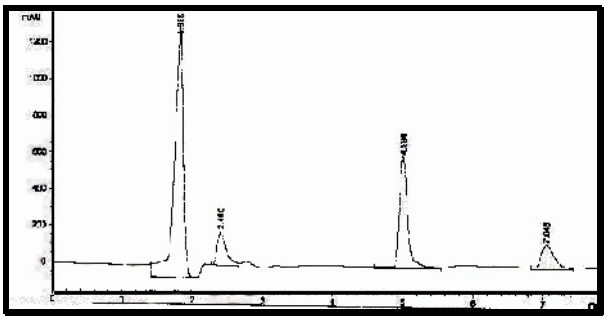
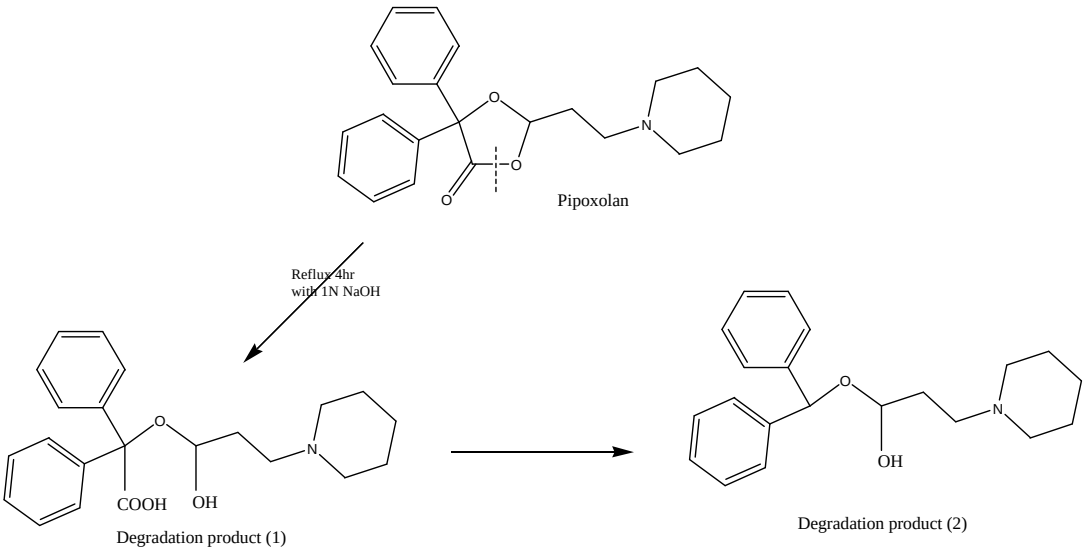


Fig. 1: The chromatogram of pipoxolan HCl (4.994 min), its alkaline-induced degradation products (1.815 and 2.400 min) and drofenine HCl (7.046 min) as internal standard.

Decarboxylation of degradation product (1) gives a degradation product containing OH with less polarity than the first [degradation product (2) 2.400 min].



Drofenine HCl and propropyphenazone are present in a binary mixture. On literature survey, no literature describing the simultaneous determination of these two drugs by LC was found. Drofenine HCl and its degradation product cyclohexane phenyl acetic acid were estimated using spherisorb ODS column and mobile phase containing acetonitrile : 0.02 M sodium acetate buffer solution with 0.59% of di-n-butylamine (65:35 v/v) adjusted to pH 4.5 with acetic acid at a flow rate 1.5 ml/min and detection at 260 nm but poor peak shapes were observed for drofenine HCl and cyclohexane phenyl acetic acid (tailing factor for drofenine HCl= 4.6 and for cyclohexane phenyl acetic acid= 2.42)[7].

Several mobile phases with different ratios for their components were tried for the separation of drofenine HCl, propyphenazone and degradation product (3) such as:

- Water pH 4.5 : methanol: (25-50 : 75–50 v/v).
- Phosphate buffer pH 4.5: acetonitrile (30–70 : 70–30 v/v).
- 0.02 M phosphate buffer pH 7.5: acetonitrile (30:70, 20:80 v/v).
- 0.05 M phosphate buffer pH 3.5: acetonitrile (30-70 : 70-30).

The chosen mobile phase was 0.05 M phosphate buffer pH 3.5: acetonitrile (60:40 v/v) as it showed good separation of drofenine HCl, degradation product (3), pipoxolan HCl and propyphenazone at a flow rate of 1.5 ml/min and UV detection at 215 nm. Retention time for drofenine HCl was 7.010 min, for degradation product (3) was 13.807 min and for propyphenazone was 3.645 min pipoxolan HCl was chosen as internal standard as it eluted at reasonable retention time at 4.564 min and showed good separation from mixture components (Fig. 2).

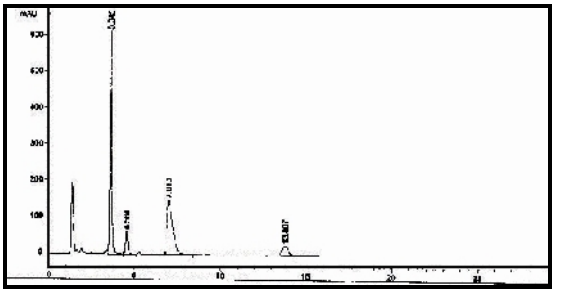


Fig. 2: The chromatogram of propyphenazone (3.645 min), pipoxolan HCl (4.564 min) as internal standard drofenine HCl (7.010 min) and its alkaline-induced degradation product (13.807 min).

Isopropamide iodide and triflouperazine HCl are present in a binary mixture. On literature survey, no literature describing the simultaneous determination of these two drugs by LC was found. Different mobile phases were tried such as:

- NaH_2PO_4 buffer pH 5.5 : acetonitrile with different ratios: (30–70 : 70–30 v/v).
- NaH_2PO_4 buffer pH 5.5: acetonitrile: methanol (50:30:20 v/v/v).
- 0.02 M Ammonium acetate buffer: acetonitrile with different ratios: (55:45 v/v).

- 0.05 M Phosphate buffer pH 3.5 : acetonitrile with different ratios: (30-70 : 70-30 v/v).
- 0.05 M Phosphate buffer pH 3.5 (containing 1ml/l triethylamine) : acetonitrile (50:50 v/v).

The chosen mobile phase was 0.05 M phosphate buffer pH 3.5 (containing 1 ml/l triethylamine): acetonitrile (50:50 v/v) as it showed good separation of isopropamide iodide and triflouperazine HCl at a flow rate of 1.5 ml/min and UV detection at 210 nm. Retention time for isopropamide iodide was 3.729 min and for triflouperazine HCl was 7.115 min. Pipoxolan HCl was chosen as internal standard as it eluted at reasonable retention time at 5.709 min and showed good separation from mixture components (Fig. 3).

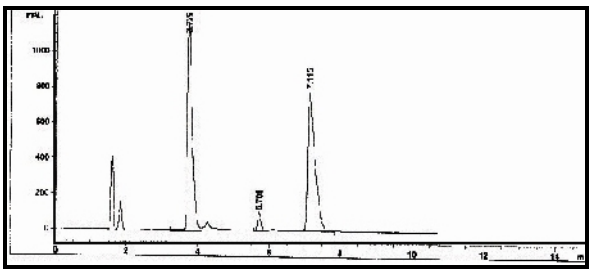


Fig. 3: The chromatogram of isopropamide iodide (3.729 min), pipoxolan HCl (5.709 min) as internal standard and triflouperazine HCl (7.115 min).

System suitability tests of LC method shows good resolution for the drugs, (Table 1).

Linear relationship was obtained between the ratios (R) of the recorded area under the peaks of each drug to that of the internal standard. The concentration ranges and the regression equations are displayed in tables 2-4.

The results of assay validation of the proposed methods show that the methods are accurate, precise and specific according to the %RSD of intraday and interday determination, (Tables 2-4).

A statistical comparison of the results obtained by the proposed methods and reference methods that depends on colorimetric determination of pipoxolan HCl[14] and drofenine HCl[15], official methods; potentiometric titration of propyphenazone[16] and triflouperazine HCl[16] and non-aqueous titration of isopropamide iodide[17].The values of the calculated t and F are less than the tabulated ones, which reveals that there is no significant difference with respect to accuracy and precision between the proposed and the reference methods, (Table 5).

Table 1: System suitability tests for the LC methods proposed for the determination of pipoxolan HCl, drofenine HCl and propyphenazone in presence of their alkaline-induced degradation products and isopropamide iodide – triflouperazine HCl binary mixture.

	Method (I)			Method (II)				Method (III)		
Item	Degradation product (2)	Pipoxolan HCl	Drofenine HCl (internal standard)	Propyphenazone	Pipoxolan HCl (internal standard)	Drofenine HCl	Degradation product (3)	Isopropamide iodide	Pipoxolan HCl (internal standard)	Triflouperazine HCl
N(number of theoretical plates)	2167	10472	6946	6761	6721	2358	6833	4273	19164	6292
R (resolution)	12.90		7.68	4.59		6.10	10.90	10.06		5.36
α (selectivity)	2.08		1.41	2.08		1.54	1.97	1.53		1.25
K (capacity factor)	----	1.774	2.914	1.69	2.37	4.19	9.22	1.04	2.12	2.89
T (tailing factor)	----	1.330	1.400	1.10	1.20	1.49	1.03	1.30	1.00	1.14

Table 2: Assay validation results obtained by the proposed LC method for the determination of pipoxolan HCl in presence of its alkaline-induced degradation products.

Item	Pipoxolan HCl		
Retention time	4.994min ± 0.119		
Wavelength of detection	215nm		
Linearity range	10 – 60 µg/ml		
Regression equation	$R = 0.1638\ C_{(\mu g/ml)} + 0.1313$		
Determination coefficient (r^2)	$r^2 = 0.9966$		
Standard deviation of slope (S_b)	4.78×10^{-3}		
Standard deviation of intercept (S_a)	0.186		
Limit of detection (LOD)	1.750		
Limit of quantitation (LOQ)	5.860		
Confidence limit of the slope	0.1638 ± 0.01327		
Confidence limit of the intercept	0.1313 ± 0.5163		
Standard error of estimation	0.200		
Intra-day			
Mean of concentrations (µg/ml) n=3	15.03	30.10	49.17
S.D.	0.400	0.829	0.377
% RSD	0.398	0.826	0.383
Inter-day			
Mean of concentrations (µg/ml) n=3	15.02	29.96	49.41
S.D.	0.637	0.737	0.312
% RSD	0.636	0.738	0.316
Results			
1) Drug in bulk	100.37 ± 1.101		
2) Drug in laboratory prepared mixture	99.87 ± 0.779		
3) Drug in dosage form	96.07 ± 0.940		
4) Drug added	99.92 ± 0.771		

Table 3: Assay validation results obtained by the proposed HPLC method for the determination of drofenine HCl and propyphenazone in presence of drofenine alkaline-induced degradation product.

Item	Drofenine HCl			Propyphenazone		
Retention time	7.010 min ± 0.089			3.645 min ± 0.040		
Wavelength of detection	215nm			215 nm		
Linearity range	20 – 140 µg/ml			10 – 70 µg/ml		
Regression equation	$R = 0.0929\ C_{(\mu g/ml)} - 0.0014$			$R = 0.2065\ C_{(\mu g/ml)} + 0.1957$		
Determination coefficient (r^2)	$r^2 = 0.9992$			$r^2 = 0.9989$		
Standard deviation of slope (S_b)	1.186×10^{-3}			3.061×10^{-3}		
Standard deviation of intercept (S_a)	0.105			0.136		
Limit of detection (LOD)	3.423			1.923		
Limit of quantitation (LOQ)	11.410			6.392		
Confidence limit of the slope	0.0929 ± 0.0030			0.2065 ± 0.0078		
Confidence limit of the intercept	0.0014 ± 0.2699			0.1957 ± 0.3519		
Standard error of estimation	0.125			0.162		
Intra-day						
Mean of concentrations (µg/ml) n=3	39.69	80.56	119.78	19.95	39.96	59.98
S.D.	0.412	0.202	0.227	0.381	0.240	0.121
% RSD	0.415	0.201	0.227	0.381	0.240	0.121
Inter-day						
Mean of concentrations (µg/ml) n=3	39.87	79.91	119.35	20.04	39.76	59.85
S.D.	0.559	0.429	0.311	0.354	0.345	0.127
% RSD	0.561	0.429	0.313	0.353	0.347	0.127
Results						
1) Drug in bulk	100.44 ± 0.905			100.15 ± 0.798		
2) Drug in laboratory prepared mixture	99.48 ± 1.283			-------		
3) Drug in dosage form	105.72 ± 1.159			107.72 ± 0.961		
4) Drug added	99.67 ± 0.685			99.85 ± 0.733		

Table 4: Assay validation results obtained by the proposed LC method for the simultaneous determination of isopropamide iodide and triflouperazine HCl in mixture.

Item	Isopropamide iodide		Triflouperazine HCl		
Retention time	3.729 min ± 0.043		7.115 min ± 0.085		
Wavelength of detection	210 nm		210 nm		
Linearity range	10 – 60 µg/ml		10 – 60 µg/ml		
Regression equation	$R = 0.2506\ C_{(\mu g/ml)} + 0.0773$		$R = 0.2553\ C_{(\mu g/ml)} + 0.0533$		
Determination coefficient (r^2)	$r^2 = 0.9994$		$r^2 = 0.9989$		
Standard deviation of slope (S_b)	3.131×10^{-3}		4.135×10^{-3}		
Standard deviation of intercept (S_a)	0.121		0.161		
Limit of detection (LOD)	1.387		1.497		
Limit of quantitation (LOQ)	4.624		4.990		
Confidence limit of the slope	0.2506 ± 0.0086		0.2553 ± 0.0114		
Confidence limit of the intercept	0.0773 ± 0.3358		0.0533 ± 0.4469		
Standard error of estimation	0.131		0.173		
Intra-day					
Mean of concentrations (µg/ml) n=3	20.00	40.01	19.88	39.94	60.05
S.D.	60.02		0.400	0.487	0.230
% RSD	0.200	0.152	0.402	0.488	0.230
	0.070				
	0.200	0.151			
	0.069				
Inter-day					
Mean of concentrations (µg/ml) n=3	20.08	39.89	19.84	40.13	59.58
S.D.	60.00		0.721	0.300	1.116
% RSD	0.200	0.305	0.726	0.299	1.124
	0.166				
	0.199	0.305			
	0.166				
Results					
1) Drug in bulk	99.55 ± 1.185		99.29 ± 0.902		
2) Drug in dosage form	97.68 ± 0.793		97.94 ± 0.765		
3) Drug added	99.73 ± 1.210		99.89 ± 0.387		

Table 5: Tests of significance for the LC methods proposed for the determination of pipoxolan HCl, drofenine HCl and propyphenazone in presence of their alkaline degradation products and isopropamide iodide - triflouperazine HCL binary mixture.

	Method (I)		Method (II)				Method (III)			
Statistical term	Pipoxolan HCl		Drofenine HCl		Propyphenazone		Isopropamide iodide		Triflouperazine HCl	
	Reference method[a]	Proposed method	Reference method[b]	Proposed method	Reference method[c]	Proposed method	Reference method[d]	Proposed method	Reference method[e]	Proposed method
Mean	100.33	100.37	99.84	100.44	99.51	100.15	100.04	99.55	99.50	99.29
S.D. ±	0.587	1.101	0.584	0.905	0.795	0.798	0.788	1.185	0.584	0.902
S.E. ±	0.240	0.449	0.238	0.369	0.324	0.325	0.321	0.483	0.238	0.368
%RSD	0.585	1.096	0.584	0.901	0.798	0.796	0.787	1.190	0.586	0.908
n	6	6	6	6	6	6	6	6	6	6
v	0.344	1.212	0.341	0.819	0.632	0.636	0.620	1.404	0.341	0.813
t(2.228)*	0.078		1.791		1.394		0.844		0.478	
F(5.050)*	3.523		2.401		1.006		2.264		2.384	

*Figures in parentheses are the theoretical t and F values at (p = 0.05).
[a]Spectrophotometric manufacturer's method(Amoun pharmaceutical Co.)[14].
[b]Spectrophotometric manufacturer's method (Pharco Co.)[15].
[c]Potentiometric method[16] [d]Non-aquous titration method[17] [e]Potentiometric method[16].

Conclusion

The proposed methods enables simple, accurate and reproducible quantitative RP-LC determinations of pipoxolan HCl in presence of its alkaline degradation products, a mixture of drofenine HCl and propyphenazone in presence of drofenine alkaline degradation product and isopropamide iodide and triflouperazine HCl binary mixture.

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