ORIGINAL ARTICLE

Rapid Analytical Method Development and Validation of Combined Tablet of Drotaverine Hydrochloride and Piroxicam

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Abstract

Introduction: Renal colic is a painful condition in which patients suffer severe flank pain which occurs as a result of obstruction of urinary tract through calculus. The treatment requires both spasmolytic and analgesic action in addition to anti-inflammatory action. **Materials and Methods:** Two drugs were used drotaverine hydrochloride (HCl) and piroxicam in one tablet as fixed dose combination. The ultraviolet spectrophotometer method was developed for assay of both drugs. **Results:** The results of method of validation for drotaverine HCl showed that all the parameters of performance were well in limit. Relative standard deviation (RSD) is 0.949% which was well under the limit of 2%. **Conclusion:** The results of method validation for piroxicam showed that all the parameters of performance were well in acceptance limit, for example, in precision criteria the repeatability test was ok. RSD was 0.582% which was well below the limit of 2%.

Key words: Analytical method, drotaverine hydrochloride, piroxicam, quality control

INTRODUCTION

Piroxicam (3E)-3-[hydroxy-(pyridine-2-yl amino)methylidene]-2-methyl-1, 1 dioxobenzo[e]thiazin-4-one (C15H13N3O4S). PXM belongs to a class of drugs known as Nonsteroidal Anti-inflammatory Drugs. Piroxicam reduces hormones that cause pain and inflammation in body. Therefore, piroxicam is specially recommended to patients suffering from osteoarthritis and rheumatoid arthritis as it reduces pain, stiffness, and inflammation in such patients.[1,2] Various analytical methods have been developed for quantitative assay of piroxicam individually and in combination medicines. The research papers of these methods also describe the validation of these methods.[3] The word validation means assessment of validity or action of providing effectiveness.

Drotaverine hydrochloride (HCl) is an antispasmodic drug. Chemically, it is 1-[(3, 4-diethoxyphenyl) methylene]-6, 7-diethoxy -1, 2, 3, 4, - tetrahydroisoquinoline HCl. Literature review revealed the development of only few analytical methods such as potentiometry, spectrophotometry, high-performance liquid chromatography in pharmaceutical formulations and in biological fluids.[4,5] In the present investigation, a ultraviolet (UV) visible spectrophotometer method has been developed for the quantitative determination of drotaverine HCl and piroxicam combined dosage form with the aim of quantification of the drug substance with highest selectivity, precision and accuracy and analytical method validation for the proposed method as per ICH Q2 (R1) guideline.[6]

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MATERIALS AND METHODS

Material

Active pharmaceutical ingredients used to formulate fixed dose combination (FDC) are drotaverine HCl and piroxicam both acquired from Harmann Pharmaceuticals as a gift. Other excipients are lactose, starch (Rafhan Maize), sodium starch glycolate (primogel), magnesium stearate, gelatin, and talcum. All excipients used are of pharmaceutical grade.

Equipments

Most of the manufacturing and testing equipment was of laboratory scale to prepare tablets on trial basis. The laboratory facilities and equipment with lab material were provided by Harmann Pharmaceutical Laboratories Pvt. Ltd.

Testing equipments include weighing balance, Fourier-transform infrared, dissolution apparatus, and UV-visible spectrophotometer. These equipments are made by Bruker Germany, Galvano Scientific, Hitachi Japan, Sartorius, Galvano Scientific.

Quantitative assay of piroxicam

Preparation of test sample

Take 20 tablets containing 20 mg of piroxicam and find average fill per tablet. Dissolve with 0.1 M NaOH in a 10 ml flask and make up the volume with same solvent. Filter the solution, dilute 5 ml of filtrate with 50 ml of some solvent.

Preparation of reference standard

Weigh accurately 20 mg of piroxicam and make same dilutions as for test sample using the same solvent.

Procedure

The absorbance of the resulting solution is measured at 334 nm. 0.1 M NaOH was taken as blank in the reference cell and the result was calculated by comparison.

$$\%\,\text{Age assay} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 100$$

Quantitative assay of drotaverine HCl

Preparation of test sample

622.5 mg weight of tablets is taken which is equal to 100 mg of drotaverine HCl in 100 ml volumetric flask. Add ethanol into flask to make up the volume.10 ml of the resulting solution is taken into another 100 ml flask and volume was made up with distill water.

Preparation of reference standard

Drotaverine HCl is accurately weighed 100 mg and dilutions are made as for test using the same solvent.

Procedure

The absorbance of the resulting solutions is measured at 242 nm taking water as blank in the reference cell and calculates the results by comparison.

$$\%\,\text{Age assay} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 100$$

Method validation of piroxicam 20 mg and drotaverine HCl 40 mg tablet

UV spectrophotometer

Simple and cost-effective UV-spectrophotometer method is developed for the estimation of drotaverine HCl and piroxicam in FDC tablet formulation.[7]

Linearity of drotaverine HCl and piroxicam

The linear relationship exists between the concentration and the instrument response as absorbance at 242 nm (for drotaverine HCl) and 334 nm (for piroxicam) is confirmed by relating the concentrations and absorbance in the range of 80, 90, 100, 110, and 120% of the target value. The results obey Beer's law.

Precision of drotaverine HCl 40 mg piroxicam 20 mg

An investigation of precision is conducted for validation purposes to further test the assay. This is tested by repeatability and reproducibility.

Repeatability

Six samples are drawn and test solutions are prepared and tested according to the test procedure.

Parameter	Drotaverine HCl 40 mg	Piroxicam 20 mg
Weight of RS*	100 mg purity 100%	20 mg, purity 100%
Absorbance of RS	2.008	679
Average weight of tablet	249 mg	249 mg
Range	98.36–100.70%	101.18–102.65%
Mean	99.3%	101.77%
Standard deviation	0.947	0.580%
RSD	0.949±2.0%	0.582%

*RS =Reference standard. RSD: Relative standard deviation

Reproducibility

To check the repeatability, 2 sets of five samples equivalent to 100% of label claim are prepared and assayed by two analysts individually.

Reproducibility of drotaverine HCl 40 mg

	Analyst 1	Analyst 2
Weight of RS	100 mg purity: 100%	100 mg, purity 100%
Absorbance of RS	2.007	2.004
Average weight of tablet	249 mg	249 mg
Mean	98.91%	99.68%
Standard deviation	0.426	0.467

HCl: Hydrochloride

Reproducibility of piroxicam 20 mg

	Analyst 1	Analyst 2
Weight of RS	20 mg purity: 100%	20 mg, purity 100%
Absorbance of RS	658	699
Average weight of tablet	249 mg	249 mg
Mean	100.94%	101.46%
Standard deviation	1.273%	0.861%

Robustness of drotaverine HCl 40 mg and piroxicam 20 mg

It is the measure of stability of the test procedure under slight variation in test procedure. The changes deliberately made in testing procedure are; the test solution prepared according to the test procedure and kept at 15°C and 35°C for 4 h and assayed according to the test procedure. The results of both drotaverine HCl and piroxicam 20 mg must be reproducible with a slight variation in temperature.

Range of drotaverine HCl 40 mg and piroxicam 20 mg

Based on linearity results test, range of analytical control procedure for assay is conformed as 80–120% of target.

Specificity drotaverine HCl 40 mg and piroxicam 20 mg

Specificity of the analytical control procedure of assay of drotaverine 40 mg tablet piroxicam 20 mg is determined by taking absorbance of the placebo sample. The absorbance obtained was 0.00.

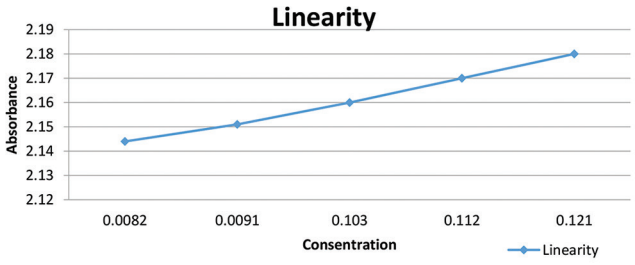
RESULTS

Method validation of product piroxicam 20 mg, and drotaverine 40 mg tablet UV spectrophotometer

Linearity (drotaverine HCl 40 mg)

Weight of active	Concentration (mg/ml)	Absorbance of test solution
82	0.0082	2.144
91	0.0091	2.151
103	0.103	2.160
112	0.112	2.170
121	0.121	2.180

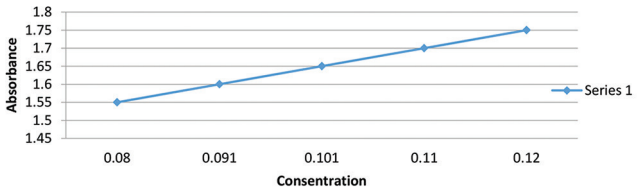
HCl: Hydrochloride



(Linearity of drotaverine HCl)

Linearity of piroxicam 20 mg

Weight of active mg	Concentration (mg/ml)	Absorbance of test solution
80	0.08	1.55
91	0.09	1.60
101	0.10	1.65
110	0.11	1.66
120	0.12	1.71

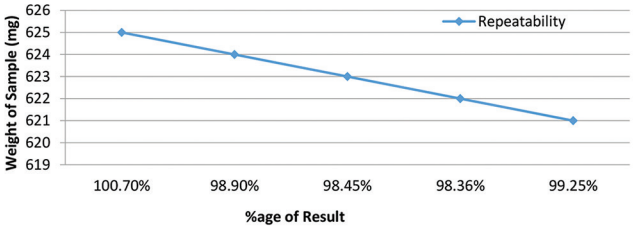


(Linearity of piroxicam 20 mg)

Repeatability (drotaverine HCl 40 mg)

Weight of sample in mg	Absorbance of sample	% age results of LC	Variation from theoretical results
625	2.022	100.70%	−0.70%
624	1.986	98.90%	1.10%
623	1.977	98.45%	1.55%
622	1.975	98.36%	1.64%
621	1.994	99.25%	1.10%

HCl: Hydrochloride

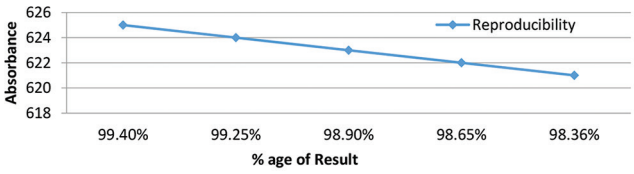


(Repeatability [drotaverine HCl 40 mg])

Reproducibility (drotaverine HCl 40 mg)

Weight of sample	Absorbance of sample	% Age results of LC	Variation from theoretical results
625	1.995	99.40%	0.60%
624	1.992	99.25%	0.75%
623	1.985	98.90%	1.10%
622	1.980	98.65%	1.35%
621	1.974	98.36%	1.64%

HCl: Hydrochloride

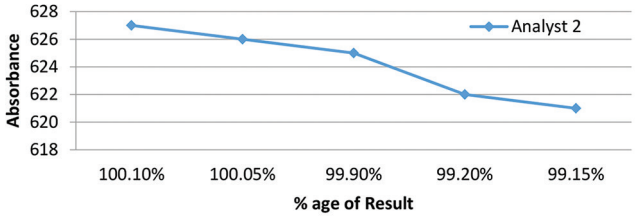


(Reproducibility [drotaverine HCl 40 mg])

Reproducibility (drotaverine HCl 40 mg)

Weight of sample	Absorbance of sample	% age results of LC	Variation from theoretical results
627	2.006	100.10%	−0.10%
626	2.005	100.05%	−0.05%
625	2.002	99.90%	0.10%
622	1.988	99.20%	0.80%
621	1.987	99.15%	0.85%

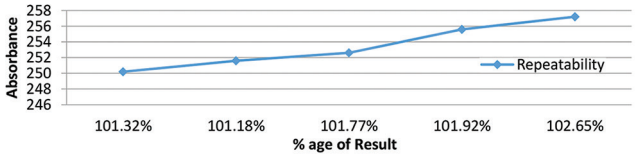
HCl: Hydrochloride



(Reproducibility [drotaverine HCl 40 mg])

Repeatability of piroxicam

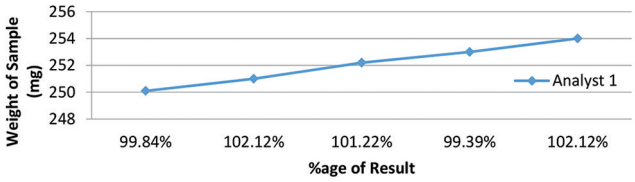
Weight of sample	Absorbance of sample	% age results of LC	Variation from theoretical results
250.2	688	101.32%	−1.32%
251.6	687	101.18%	−1.18%
252.6	691	101.77%	−1.77%
255.6	692	101.92%	−1.92%
257.2	697	102.65%	−2.65%



(Repeatability [piroxicam 20 mg])

Reproducibility (piroxicam 20 mg)

Weight of sample mg	Absorbance of sample	% Age results of LC	Variation from theoretical results
249	657	99.84%	0.16%
251	672	102.12%	2.12%
250	666	101.22%	−1.22%
252	654	99.39%	0.07%
253	672	102.12%	−2.12%



(Reproducibility [piroxicam 20 mg])

Reproducibility (piroxicam 20 mg)

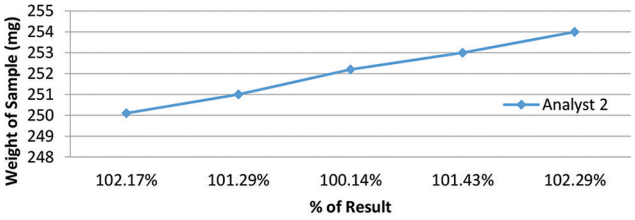
Weight of sample	Absorbance	% age results of LC	Variation from theoretical results
250.1	718	102.17%	−2.71%
251.0	708	101.29%	−1.29%
252.2	700	100.14%	−0.14%
253.0	709	101.43%	−1.43%
254.0	715	102.29%	−2.29%

Robustness of drotaverine 40 mg

Storage condition	15°C		25°C		35°C	
	Sample I	Sample II	Sample I	Sample II	Sample I	Sample II
Weight of samples	623	626	623	626	623	626
Abs. of test solution	1.985	2.019	1.985	2.011	1.980	1.996
% of Label claim	98.71	100.40	98.71	100.00	98.46	99.25
Average (%)	99.56		99.36		98.86	
Standard deviation	1.20		0.91		0.56	

Robustness of piroxicam 20 mg

Storage condition	15°C		25°C		35°C	
	Sample I	Sample II	Sample I	Sample II	Sample I	Sample II
Weight of samples	250	251	250	251	250	251
Abs. of test solution	710	716	707	695	704	709
% of label claim	101.28	102.14	100.86	99.14	100.43	101.14
Average (%)	101.17		100.0		100.79	
Standard deviation	0.608		1.216		0.502	



(Reproducibility [piroxicam 20 mg])

DISCUSSION

Method validation

Linearity of drotaverine HCl 40 mg

R^2=0.904869 which shows very good positive relation.[8]

Linearity of piroxicam 20 mg

R^2=0.983783 which shows very good positive relation.[9]

Repeatability of drotaverine HCl 40 mg

$P = 0.03728 \leq 0.05$ which shows that experimental results are precise and difference with theoretical values is in significant.[10]

Reproducibility drotaverine HCl 40 mg

$P = 0.013201 \leq 0.05$ which shows that experimental results for drotaverine are reproducible.[11]

Repeatability of piroxicam 20 mg

$P = 0.000068 \leq 0.05$ which shows that experimental results are precise and difference with theoretical values is in significant.[9]

Performance parameters acceptance limit and results (Drotaverine HCl 40 mg)

Parameters	Acceptance limit	Result	Remarks
Precision	Repeatability RSD NMT% Reproducibility Result should reproducible by different analysts with standard deviation NMT 2%	0.949 0.426 0.467	OK
Accuracy	For assay: ±2% of theoretical concentration	Complies	OK
Robustness	Results should be reproducible at slight variation in temperature	Complies	OK

RSD: Relative standard deviation

Reproducibility piroxicam 20 mg

$P = 0.2329 \geq 0.05$ which shows that experimental results are not reproducible.[9]

Robustness (drotaverine 40 mg)

The test samples were stable for up to 4 h kept under the temperature as low as 15°C and as high as 35°C. The results of standard deviation was 1.20 and 0.56, respectively.[11]

Robustness (piroxicam 20 mg)

It was the measure how stable the test procedure was under slight variation in test procedure. The following changes were made deliberately in testing procedure. The test samples were stable for up to 4 h kept under the temperature as low as

Performance parameters acceptance limit and result (piroxicam 20 mg)

Parameters	Acceptance limit	Result	Remarks
Precision	Repeatability RSD NMT 2% Reproducibility Result should reproducible by different analysts with standard deviation NMT 2%	0.582% 1.273% 0.861%	OK
Accuracy	For assay: ±2% of theoretical concentration	Complies	OK
Robustness	Results should be reproducible at slight variation in temperature	Complies	OK

RSD: Relative standard deviation

15°C and as high as 35°C.[9] The results of standard deviation were 0.608 and 0.502, respectively, which was well under the limit 2%.

CONCLUSION

The FDC of drotaverine HCl and piroxicam, which have been evaluated successfully for the first time in the form of single tablet, as results of all analytical tests comply within the pharmacopoeial limits. The values of RSD are <2.0% indicating the accuracy and precision of the method. Furthermore, repeatability and robustness were observed in method as the RSD in these tests were also below 2.0%.

Future prospect

This method provides the quantitative assessment of combination tablet of piroxicam and drotaverine HCl. Further bioavailability and pharmacokinetic evaluation of this formulation is recommended for confirmation of safety and therapeutic efficacy of this dosage form so that that this formulation can be produced commercially for the benefit of the renal colic patients. This research will also help researchers in developing methods for other FDCs.

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