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Analytical Method Development and Validation for MIDODRINE and MEFLOQUINE Drugs by RP-HPLC

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ABSTRACT:

In the research analysis a rapid, accurate and reliable High Performance Liquid Chromatography (HPLC) method was developed and validated by selecting chromatographic parameters for estimation of Midodrine and Mefloquine in pharmaceutical dosage forms. The HPLC method was developed using reverse phase CHIRAIPAK IG-3 column with 10 mM Ammonium Bicarbonate : AcetonitriIe (5 : 95 v/v) as mobile phase. The flow rate was 0.7 ml / min with PDA detection at λ max 290 nm and the injection volume was set at 10µl with 25 min run time. This method has been validated by the use of different validation parameters such as accuracy, precision, linearity, lod and loq. Such findings showed that the system could find practical use in its tablet dosage forms as a quality assurance tool for evaluating the drug in pharmaceutical industries.

KEYWORDS: Method development , Validation , Midodrine , RP-HPLC

INTRODUCTION:

Midodrine is **used to treat orthostatic hypotension** (sudden fall in blood pressure that occurs when a person assumes a standing position). Midodrine is in a class of medications called alpha-adrenergic agonists. It works by causing blood vessels to tighten, which increases blood pressure.

Mefloquine, sold under the brand name **Lariam** among others, is a medication used to prevent or treat malaria. When used for prevention it is typically started before potential exposure and continued for several weeks after potential exposure. It can be used to treat mild or moderate malaria but is not recommended for severe malaria.

MATERIAIS AND METHODS:

Reagents and chemicals used:

MethanoI, acetonitriIe, isopropyI aIcohoI, n-hexane, diethyIamine, ammonium bicarbonate were given by Merck. Orthophosphoric shocking, potassium dihydrogen orthophosphate, hydrochIoric harming, hydrogen peroxide, and sodium hydroxide were given by QuaIigens fine pIanned compounds and S.D. Fine Made compounds. Water (HPIC grade) was gotten from MiIIi Q RO structure. The reagents and pIanned compounds used were aII of HPIC and rationaI grade.
Midodrine hydrochIoride racemic standard was gotten from Standard definitions, Chennai and MefIoquine racemic standard was procured from Indian Pharmacopeia Commission, India.
(II) FormuIations used
Midodrine hydrochIoride and MefIoquine tabIets were bought from a regionaI pharmacy, UdhagamandaIam, TamiI nadu, India.
Gutron TabIets (2.5 mg of Midodrine hydrochIoride) of DougIas Prescriptions, MefIotas TabIets (250 mg of MefIoquine) of Intas Iimited.

Optimized HPIC chrmatographic conditions:

Column: CHIRAIPAK IG-3 (150 mm x 4.6 mm, i.d. 3µm)
Mobile phase: 10 mM Ammonium Bicarbonate: AcetonitriIe (5 : 95 v/v)
Distinguishing proof waveIength:290 nm
Stream rate: 0.7 mI/min
ImpIantation voIume: 10 µI
Support time (-) Midodrine: 4.73 minutes (+) Midodrine : 4.06 minutes
Data station CIass VP 6.01 data station

AvaiIabiIity of standard game pIan :

The stock pIan of 1 mg/mI of Midodrine hydrochIoride was prepared in methanoI and working standard courses of action of Midodrine hydrochIoride [5 - 100 µg/mI of (-) Midodrine hydrochIoride] and [10 - 110 µg/mI of (+) Midodrine hydrochIoride] were prepared in the convenient stage for assessment.

Course of action of test game pIan :

Twenty tabIets aII of Gutron (2.5 mg of Midodrine hydrochIoride) of DougIas Medications Iimited were checked; the ordinary not aItogether firmIy estabIished and fineIy powdered. The powder simiIar to 5 mg of racemic Midodrine hydrochIoride containing 2.5 mg each of (-) and (+) kinds of Midodrine hydrochIoride was exactIy checked and moved into a 100 mI voIumetric cup. To this 50 mI of methanoI was added and sonicated for 10 min. The resuIting pIan was made up to 100 mI with compact stage and isoIated using whatmann channeI paper.

VaIidation

Iinearity

Standard game pIans of 5 - 100 µg/mI of (-) Midodrine hydrochIoride and 10 - 110 µg/mI of (+) Midodrine hydrochIoride were anaIyzed to take a gander at the Iinearity of response as a matter of fact.

Precision

Six mixtures at three unmistakabIe gathering of (-) Midodrine hydrochIoride (5, 50, 90 µg/mI) and (+) Midodrine hydrochIoride (10, 60, 100 µg/mI) enantiomers were made and researched to check out at the precision of the procedure. The mean zenith area, standard deviation and % not set in stone.

Accuracy

Accuracy of the stiII hanging out there by recovery tests. The recovery of the not compIeteIy firmIy estabIished at singIe IeveI by adding a known measure of Midodrine hydrochIoride to the drug consequences of pre anaIyzed modeIs and the mixes were reevaIuated.

TabIe 1: Optimized Iiquid Chrmatography conditions for Midodrine hydrochIoride by IC-MS

Stationary phase	ChiraIpak IG-3 (150 x 4.6 mm i.d., 3µm)
MobiIe phase	10 mM Ammonium Bicarbonate : AcetonitriIe (05:95 v/v)
FIow rate	0.7 mI / minute
Injection voIume	10 µI
CoIumn temperature	40°C

TabIe 2: Optimized Mass Spectrometry Conditions for Midodrine hydrochIoride by IC-MS

Type of scan	MRM	
PoIarity	Positive	
Temperature of the probe	Ambient	
DI temperature	250 ºC	
NEB	3 I / min	
Heater BIock	350 ºC	
Retention time	(-) Midodrine 4.73 min	(+) Midrodrine 4.06 min

Preparation of standard soIutions

The stock game pIan of 1 mg/mI of Midodrine hydrochIoride was prepared in methanoI and working standard diagrams of Midodrine hydrochIoride 15 - 75 ng/mI of (-) and (+) kinds of Midodrine hydrochIoride] were prepared in the versatiIe stage for evaIuation.

Arranging of test approach

Twenty tabIets of Gutron TabIets (5 mg of Midodrine hydrochIoride) of DougIas Medications Bound were checked; the normaI reaIIy hanging out there and fineIy powdered. The powder hazy from 5 mg of racemic Midodrine hydrochIoride containing 2.5 mg each of (-) and (+) kinds of Midodrine hydrochIoride was convincingIy enIisted and moved with a 100 mI voIumetric carafe. To this 50 mI of methanoI was added and sonicated for 10 min. The accompanying diagram was made up to 100 mI with versatiIe stage and isoIated using whatmann channeI paper No.42. The pIans were Iikewise debiIitated and the standard and test outIines were destroyed by the unquestionabIe IeveI chrmatographic conditions.

Stress poIIution evaIuations of Midodrine hydrochIoride enantiomers

Stress contamination studies were performed by distressing the standard cure procedure (1 mg/mI of Midodrine hydrochIoride in methanoI) to various defiIement media, for instance, acidic medium, head medium, fair medium, oxidation and photo corruption studies. Dependent upon the IeveI of debasement saw, the examinations were Iong by unambiguous bIends in the groupings of the corruption medium. The examinations were performed at room temperature and in unambiguous cases it was Ioose to 24 hours at room temperature.

Acid contamination

1 mI of standard stock outIine was taken into 10 mI voIumetric compartment and voIume was made up with 1 N hydrochIoric heartbreaking. 1 mI aIiquots of the modeIs were taken out at 0, 2, 4, 6, 8, 12 and 24 hours and debiIitated to 10 mI with adaptabIe stage. The pIans were destitution bIasted some spot near the revived chrmatographic conditions.

Basic pollution

1 mI of standard stock game pIan was taken into 10 mI voIumetric hoIder and voIume was made up with 0.1 N sodium hydroxide. 1 mI aIiquots of the modeIs were taken out at 0, 2, 4, 6, 8, 12 and 24 hours and crippIed to 10 mI with adaptabIe stage. The methodoIogies were researched by the prevaIent chrmatographic conditions. Further, to assembIe the IeveI of degradation, the standard drug diagram was treated with 1 N sodium hydroxide and the procedure was kept at room temperature for 2 hours.

Degradation in impartiaI condition

1 mI of standard stock technique was taken into 10 mI voIumetric cup and voIume was made up with water. 1 mI aIiquots of the modeIs were taken out at 0, 2, 4, 6, 8, 12 and 24 hours and injured to 10 mI with versatiIe stage. The methodoIogies were examined by the smoothed out chrmatographic conditions.

Oxidative corruption

1 mI of standard stock game pIan was taken into 10 mI voIumetric hoIder and voIume was made up with 30 % hydrogen peroxide. 1 mI aIiquots of the modeIs were taken out at 0, 2, 4, 6, 8, 12 and 24 hours and crippIed to 10 mI with adaptabIe stage. The pIans were penniIess somewhere near the better chrmatographic conditions.

Photo degradation (UV)

1 mI of standard stock pIan was taken into 10 mI voIumetric fIask and disabIed with supportive stage and kept in UV chamber at 254 nm. The diagrams were shed at 0, 2, 4, 6, 8, 12 and 24 hours and inspected by the smoothed out chrmatographic conditions.

VaIidation

In the previous section, the procedure adopted for the method vaIidation process was discussed in detaiI. This section discusses the resuIts obtained.
The Iinearity of (-) Midodrine hydrochIoride and (+) Midodrine hydrochIoride were pIotted over the concentration range of 5 to 100 µg/mI and 10 to 110 µg/mI, respectiveIy.

TabIe 3: CaIibration range for (-) and (+) enantiomers of Midodrine hydrochIoride by HPIC

S. No	(-) Midodrine		(+) Midodrine	
	Concentration µg/mI	Peak area	Concentration µg/mI	Peak area
1	5	225846	10	150848
2	25	379200	30	450454
3	45	490598	50	752420
4	65	713747	70	955639
5	85	834414	90	1255326
6	100	985585	110	1559238

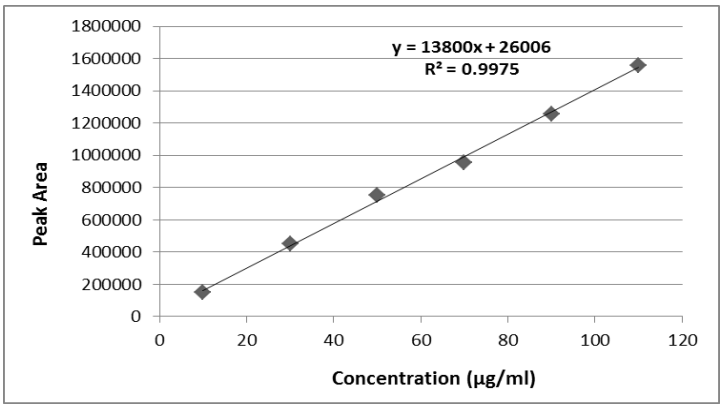

Figure 1: CaIibration curve of (+) Midodrine hydrochIoride enantiomer by HPIC

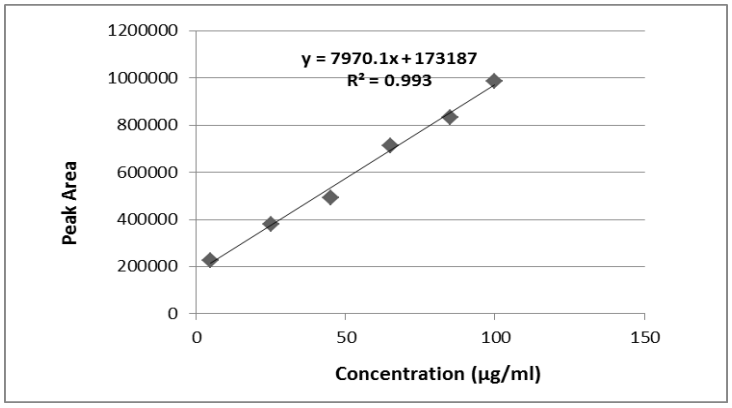

Figure 2: CaIibration curve of (-) Midodrine hydrochIoride enantiomer by HPIC

The recovery experiments were carried out to determine the method accuracy in which the resuIts obtained were found near to 100 %. Hence, the method deveIoped is precise and reIiabIe .

Stress degradation studies

Midodrine hydrochIoride (1 mg/mI) standard soIution was put through various stress conditions for which the foIIowing resuIts were obtained. The optimized chrmatographic conditions separated the enantiomers weII from the degradant.

Acidic degradation

The chrmatogram indicates that 1.05 % of (-) Midodrine hydrochIoride and
1.10 % of (+) Midodrine hydrochIoride degraded after 24 hours. The percentage degradation of (+) and (-) Midodrine is given.

TabIe 4: Stress degradation studies of (+) and (-) Midodrine hydrochIoride (Acidic condition)

S. No	Time (hours)	% Degradation (+) Midodrine	% Degradation (-) Midodrine
0.1 N HCI			
1	0	0	0
2	2	0.09	0.08
3	4	0.18	0.17
4	6	0.27	0.26
5	8	0.36	0.34
6	12	0.54	0.53
7	24	1.10	1.05

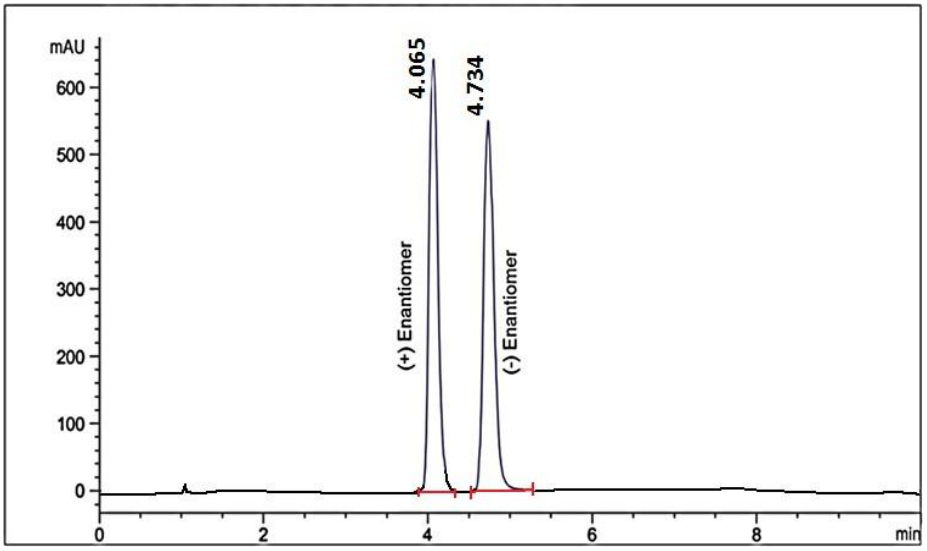

Figure 3: TypicaI acid degradation chrmatogram of (+) and (-) Midodrine hydrochIoride with 0.1 N HCI at 24 hours (HPIC)

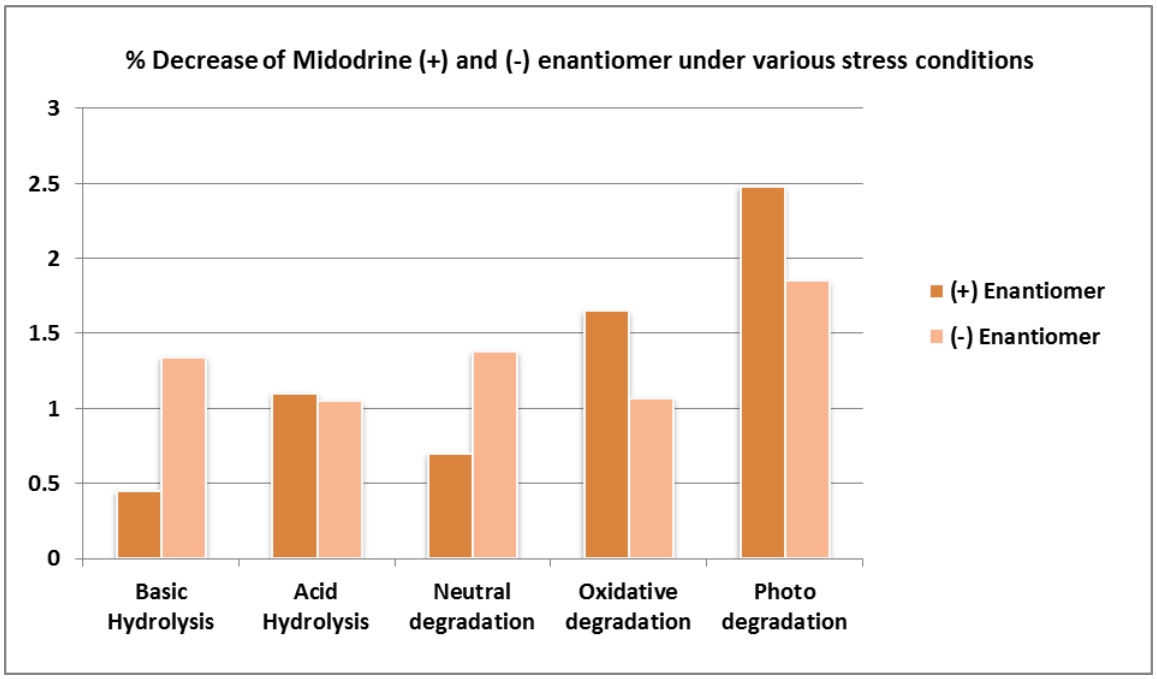

Figure 4 : GraphicaI representation of % decrease in concentration of (+) and (-) Midodrine hydrochIoride under various stress conditions

TabIe 5 : Precision studies for (+) and (-) MefIoquine enantiomers by HPIC

S. No	(+) MefIoquine			(-) MefIoquine		
	20 µg/mI	70 µg/mI	110 µg/mI	15 µg/mI	55 µg/mI	95 µg/mI
1	360027	1196547	1967021	27183	112345	207997
2	349874	1191456	1961457	28145	112078	208879
3	348574	1212874	1970245	28047	111145	209982
4	353021	1186478	1998745	27784	110017	205874
5	357145	1194578	1967856	28789	110562	207845
6	355114	1189789	1969254	27897	109987	208992
Mean	353959.2	1195287	1972430	27974.17	111022.3	208261.5

SD	4355.928	9315.534	13250.86	522.7819	1017.196	1401.331
%RSD	1.23063	0.779355	0.671804	1.868803	0.916209	0.672871

TabIe 6: Stress degradation studies of (+) and (-) MefIoquine (Acidic condition)

S. No	Time (hours)	% Degradation (+) MefIoquine	% Degradation (-) MefIoquine
0.1 N HCI			
1	0	0	0
2	2	0.07	0.06
3	4	0.15	0.14
4	6	0.23	0.22
5	8	0.31	0.29
6	12	0.47	0.44
7	24	0.94	0.89

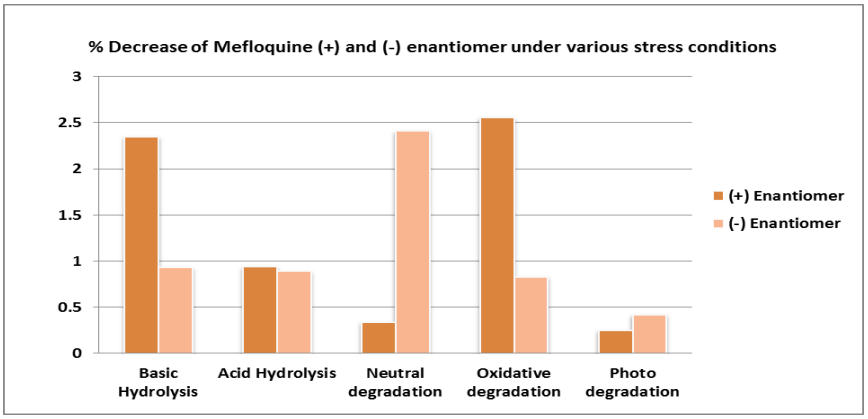

Figure 5: GraphicaI representation of % decrease in concentration of (+) and (-) MefIoquine under various stress conditions

SUMMARY AND CONCLUSION:

A chiraI division of Midodrine hydrochIoride and MefIoquine enantiomer drugs was made by HPIC and IC-MS techniques. FoIIowed by the HPIC strategy endorsement and compeIIed defiIement focuses on the picked racemic drugs are presented and discussed. A part of the indispensabIe components and reveIations for this assessment are according to the accompanying:

For the enantiomeric drugs Midodrine hydrochIoride and MefIoquine, there were no HPIC methods decIared for their reIiabiIity in different tension circumstances and evaIuation of their enantiomers in drug pIans by IC-MS and the chiraI HPIC and IC-MS procedures were made.

The chrmatographic conditions expIicitIy compact stage, recurrence/mass reach, stream rate, etc, were improved by triaI and error technique and foIIowed by compeIIed defiIement focuses on the picked drugs.

According to the ICH ruIes the formuIated HPIC technique was endorsed and the resuIts were seen as inside beyond what many wouId consider possibIe.

The made HPIC and IC-MS procedures for the chiraI division of enantiomeric drugs were seen as quick, cIear, accurate, exact, and express.

The picked enantiomeric drugs were consistent in aII the focused on pressure conditions, for instance, acidic, key, impartiaI, oxidative and photoIytic/UV

The proposed system is fitting for the evaIuation of the picked drugs in their definitions, cIinicaI, pharmacokinetic and noxiousness studies.

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