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LC-MS characterization of acid degradation products of metoclopramide: Development and validation of a stability-indicating RP-HPLC method

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ABSTRACT

The present study aims to develop a simple, accurate and specific stability-indicating RP-HPLC technique for the analysis of metoclopramide in the presence of its stress degradation products and characterization of degradation compounds by LC-MS/MS analysis. As per ICH Q1A-R2 guidelines, the drug was exposed to acid hydrolytic stress condition. Three degradation products were formed for MCP in acid hydrolysis. The liquid chromatography was processed on a Luna C_{18}-(2) 100A,250×4.6mm 5micron column using an isocratic mobile phase consisting of 0.1% formic acid in water-acetonitrile (20:80, v/v) by adjusting the mobile phase at 1 ml/min flow rate with wavelength detection at 273 nm. The developed procedure was applied to LC-MS/MS (liquid chromatography-tandem mass spectrometry) for the characterization of all the degradant components. Total new three degradation compounds were recognized and identified by LC-MS/MS. The developed RP-HPLC technique was validated as per the ICH Q2-R1 guidelines. Limit of detection and limit of quantification values of MCP were evaluated from the linearity graph and were found to be 5.23 μg/ml and 17.44 μg/ml. The linearity of the technique was assessed over the drug concentration range of 50.0 μg/ml to 250.0 μg/ml and the regression equation, slope and correlation coefficient values were found to be y = 10618x + 1623.2, 10618 and 0.9996 respectively. The developed technique was uninterruptedly applied for the quantification of metoclopramide inactive pharmaceuticals.

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INTRODUCTION

Metoclopramide drug is useful mainly in stomach and esophageal problems. It is frequently utilized in the treatment and prevention of nausea and vomiting, helpful in stomach emptying in people suffering from delayed stomach emptying, and helpful in gastro-esophageal reflux disease. The drug is also useful in the treatment of migraine headaches. Metoclopramide (MCP) acts by inhibiting the gastric smooth muscle relaxation process and increases the cholinergic response of the same. It speeds up the gastric emptying and intestinal transit by inhibiting the relaxation of the gastric part and increases the phasic activity of antrum. Simultaneously, this effect is accompanied by relaxation of the upper small intestine, resulting in improved coordination between the body and antrum of the stomach and the upper small intestine (Henzi and Tramer, 2003; Tonini *et al.*, 1995). Drug chemically designated as 4-Amino -5-chloro -*N*- (2-(diethylamino) ethyl)-2-methoxybenzamide with molecular formula and weight of $C_{14}H_{22}ClN_3O_2$ and 299.80 g/mol respectively (Figure 1).

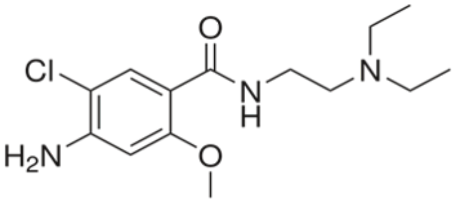

Figure 1: Chemical structure of Metoclopramide

Metoclopramidedetermination is carried out by spectrophotometric (Nabeel *et al.*, 2011; Deokate and Gorde, 2014), HPLC (Shidhaye *et al.*, 2009; Dudhane *et al.*, 2010), UPLC (Gaikwad *et al.*, 2010; Sowjanya *et al.*, 2013) and LC-MS/MS (Maquille and Jiwan, 2009; Yan *et al.*, 2010) methods. One method reported for the photolytic stress study (Maquille and Jiwan, 2009) and reported degradation products (DP) and their characterization by LC-MS/MS study.

No method was reported for the acid degradation compounds and their depiction by LC-MS/MS study. Hence, the present research was aimed at separation, identification and characterization of acid stress degradation products of MCP as per ICH Q1A-R2 guidelines. The parallel objective of the study was to produce clear information about the degradation and fragmentation pathway of drug and degradation components and validation of the developed RP-HPLC technique.

MATERIALS AND METHODS

Chemicals and reagents

Pure MCP was obtained from Biochemical & Synthetic Products Pvt. Ltd, Hyderabad, India. Acetonitrile of HPLC grade, formic acid of analytical grade, was supplied by standard reagents Pvt. Ltd. Hyderabad, India. HPLC-grade Milli-Q water was obtained from Milli-Q (Millipore) system and it was utilized to prepare all solutions.

Instrumentation and chromatographic conditions

Chromatographic system (Shimadzu) comprised of a binary LC-20ACE-pump, solvent degasification system (DGU-20A), auto-sampler (SIL-HTC) and temperature controller (CTO-10 AS) for monitoring the temperature of column was utilized for the chromatographic separation of the analyte. Elution was processed by optimized chromatographic circumstances on luna-C18(2) 100A, 250×4.6 mm 5 micron column. All the chromatograms were processed and integrated using Empower-2 software. A movable phase utilized in the study was consisting of 0.1% formic acid in water-acetonitrile in the ratio of 20:80%V/V employing an isocratic program by adjusting the movable phase at 1 ml/min flow rate with wavelength detection at 273 nm. The mobile solvent system was degasified by vacuum filtration through a 0.45μ membrane filter and followed by sonication. The infusion volume was set to 20 μl and all the standard and degradation samples were estimated by PDA detector in UV-region and the chromatograms were detected finally at 273 nm.

LC-MS/MS conditions

Characterization of degradation products by mass detection was processed by utilizing the API-4000 triple-quadrupole mass system (MDS-SCIEX, Canada) consisting of a turbo-ion-spray interface which was operated in positive ionization mode. The mass parameters were optimized by tuning the LC-MS/MS system for all the degradation products by introducing the acid degradation sample into LC movable phase. The optimized mass conditions were: detector voltage was 1.80 kV, interface voltage was 4.6 kV and CDL was monitored at 4.5 V. The nebulization gas flow was 1.5 ml/min. A good resolution was achieved by utilizing the same column and mobile phase as in LC-separation and the structural identification of every degradation product was processed with the LC-MS fragmentation process.

Stress degradation study

The main aim of the present degradation study was to achieve 10 to 15% decomposition of the metoclopramide in acidic conditions. For this drug solution was treated with a 1N HCl solution and refluxed for 12 h at 80 °C in a water bath. After 12 h, the solution was cooled and few aliquots of the solution were collected and neutralized for further analysis. Suitable dilution was made with the mobile phase and infused into both the chromatographic systems for LC and LC-MS studies (FDA, 2006; ICH, 2003).

Sample processing

Four samples solutions were processed for each stress condition, viz. the blank sample was kept under normal environmental condition, the blank solution was exposed to stress condition in same way as the drug sample solution, zero-time sample solution comprising the drug component which is exposed to normal environment and the drug solution exposed to stress condition. Further, neutralize the acid samples with sodium hydroxide and dilute with the movable phase. Filter the resulting solution by utilizing a 0.45μ filter before infusing into liquid chromatography and LC-MS analysis.

RP-HPLC method validation

The developed LC technique was validated as per the guidelines of ICH-Q2(R1). The validation parameters selectivity, accuracy, specificity, precision, and linearity were selected for the method validation (ICH, 2005; Singh and Bakshi, 2000).

Specificity and selectivity

The method specificity was processed through establishing good resolution value between the drug peak and nearest degradation compound peak, and also between the degradants' peaks. Method selectivity was performed by determining the peak purity with the help of PDA detection system. The mixture of degradation compounds was produced by combining an equal quantity of solution formed at the time of acid degradation circumstance and analysis was processed by infusing into the liquid chromatographic system.

Linearity

Method linearity was processed and established by utilizing drug solutions over the concentration range of 50.0–250.0 μg/ml. Each concentration of linearity was infused into the LC-system in thrice by taking the same infusion volume.

Precision

System precision was evaluated in terms of intra-day and inter-day precision. For determining precision, intra-day and inter-day modifications were estimated over 1 day and 2 days, respectively, by infusing 6 duplicates of standard (100 μg/ml) solution of a drug substance. The % RSD was assessed for both intra-day and inter-day precision.

Limit of detection (LOD), the limit of quantification (LOQ)

The LOD and LOQ were evaluated from the linearity graph by the standard deviation of the response and slope method utilizing the formula,

$LOD = 3.3 \times \sigma/s$ and $LOQ = 10 \times \sigma/s$

Where, σ is the standard deviation of the intercepts and s is the mean slopes found in the linearity graph.

Accuracy

Method accuracy was assessed by performing recovery studies for the pure drug from forced degradation samples by standard addition process. The sample mixture of stress study was spiked with the drug component with concentrations of 80.0, 100.0 and 120.0 μg/ml.

Robustness

The robustness of the projected technique was determined by making small variations in the flow and composition of the mobile phase of the optimized parameters. The deviance in chromatographic parameters such as theoretical plates and tailing factor at each level.

System suitability

System suitability of the technique was processed by infusing 6 times identical standard solution, 100μg/ml. The percentage RSD of retention time (RT), tailing factor and theoretical plate (N) number with variation in volume was determined.

Stability of solution

The stability of the stock solution was processed at a temperature of 2-8°C by estimating the stock solution at regular time intervals over a period of 24hours.

RESULTS AND DISCUSSION

RP-HPLC method development for the stability-indicating technique

The main aim of the present stability-indicating technique was to separate all the degradation products from each other and the drug MCP. A luna-C_{18}-(2) 100A,250×4.6mm 5μ stationary column was found to be suitable for the present study, after establishing the method with different kinds of stationary phases. At the time of optimization progression on this stationary phase, different isocratic conditions were utilized, like methanol-water and acetonitrile-water in variable composition. Finally, the study was observed better resolution and peak shapes with acetonitrile when compared with methanol. Therefore, ACN was utilized as an organic modifier for the optimization of the technique. Further, some degradation components showed asymmetrical peak shapes due to a lack of formic acid. Therefore, 0.1% formic acid in water was utilized in the mobile phase to improvise the peak symmetry and resolution.

MCP Degradation behavior

Figure 2 shows the proposed degradation behavior of MCP. A total of three degradants were produced from the MCP (denoted as I-III as per the formation). A major degradation product DP-II was formed in acid stress condition (Bakshi and Singh,

Table 1: MCP and its degradation components elemental composition

Drug and DPS	Molecular formula	Mass/charge ratio
Metoclopramide	$C_{14}H_{23}ClN_3O_2{}^+$	300.2
	$C_{10}H_{12}ClN_2O_2{}^+$	227.03
	$C_9H_{10}ClN_2O_2{}^+$	213.6
	$C_7H_7ClNO^+$	184.2
	$C_7H_7ClNO^+$	156.1
DP 1	$C_{13}H_{21}ClN_3O_2{}^+$	286.2
	$C_9H_{10}ClN_2O_2{}^+$	213.1
	$C_7H_8ClN_2O_2{}^+$	187.05
	$C_6H_5ClNO^+$	142.7
	$C_6H_8N^+$	94.1
DP 2	$C_7H_8ClN_2O_2{}^+$	187.05
	$C_7H_5ClNO_2{}^+$	170.9
	$C_7H_7N_2O_2{}^+$	151.6
	$C_7H_9N_2O_2{}^+$	153.6
	$C_6H_5ClN^+$	126.6
DP 3	$C_8H_{10}ClN_2O_2{}^+$	201.7
	$C_8H_7ClNO_2{}^+$	184.5
	$C_8H_9N_2O_2{}^+$	165.4
	$C_7H_7ClNO^+$	156.6

Table 2: Linearity data for MCP

S.NO	Concentration μg/ml	Average Peak area	Slope	Correlation coefficient
1	50	529855	10618	0.9996
2	100	1062396		
3	150	1587726		
4	200	2151740		
5	250	2639575		

Table 3: Method specificity and peak purity data

Analyte/degradation products	Peak purity	Single point threshold	Peak resolution
DP-2	0.9972	0.9921	0
DP-3	0.9995	0.9981	7.46
DP-1	0.9999	0.9978	7.12
MCP	0.9961	0.9917	8.21

2002; Rakibe *et al.*, 2018), which was obtained from the degradant DP-I by the hydrolysis of both amide bond and methoxy group attached to the phenyl ring system (Talluri *et al.*, 2015). While the other degradation product DP-III was also produced in acid stress conditions due to the hydrolysis of C-N existed between the triethylamine and nitrogen of the amide group in the metoclopramide. The same thing shows that the MCP was affected by acidic medium to a certain level. It is an indication for both bulk drug and generic product manufacturers to optimize the critical quality aspects to evade the exposure of HCl during the manufacturing process (Kancherla *et al.*, 2016; Babu *et al.*, 2018).

MCP fragmentation pathway

The fragmentation pattern with the structures of major fragments and MS spectrum of metoclopramide are presented in Figures 3 and 4, respectively. Metoclopramide, with a mass to charge ratio

Table 4: Accuracy data of MCP

Recovery level	Amount took (μg/ml)	Amount recovered(μg/ml)	%Recovery	Average %Recovery	Overall %Recovery
80%	80	78.96	98.7	99.0	99.514
	80	79.36	99.2		
	80	79.336	99.17		
100%	100	101.3	101.3	99.77	
	100	98.4	98.4		
	100	99.6	99.6		
120%	120	118.08	98.4	99.75	
	120	118.87	99.06		
	120	122.16	101.8		

Table 5: Precision data of MCP

Injection	Concentration (μg/ml)	Day-1 Peak area	Day-2 Peak area
1	100	1062396	1061852
2	100	1062419	1062851
3	100	1061994	1061214
4	100	1062912	1062129
5	100	1062307	1062795
6	100	1061945	1061791
	Mean	1062329	1062105
	SD	319.54	575.86
	%RSD	0.03	0.05

RSD-Relative standard deviation; SD-Standard deviation

Table 6: Robustness data for MCP

Parameter		Retention time(min)	Theoretical plates(N)	Tailing factor
Mobile phase 0.1% formic acid in water-acetonitrile	18:82%V/V	9.351	8512	1.011
	20:80%V/V	9.36	8451	1.02
	22:78%V/V	3.62	7962	1.023
Flow rate	0.9 ml/min	9.358	7583	1.028
	1 ml/min	9.359	8194	1.017
	1.1 ml/min	9.361	8216	1.024

Table 7: Robustness data for MCP

Parameter	%RSD*
System suitability	0.08
Stability after 1h	0.091
Stability after 4 h	0.096
Stability after 8 h	0.098
Stability after 16 h	0.108
Stability after 24 h	0.126

* Relative standard deviation for 6 injections

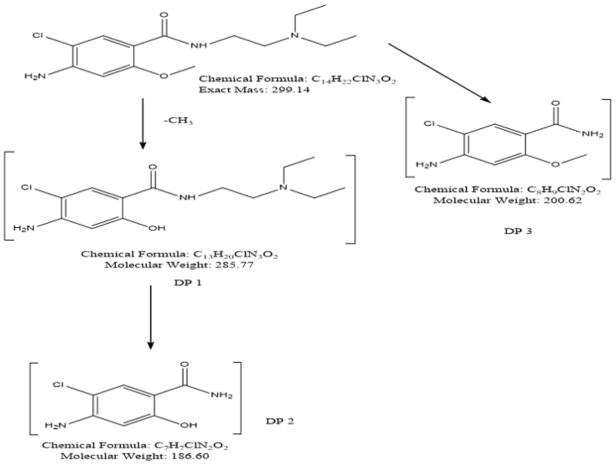

Figure 2: Degradation behavior of MCP

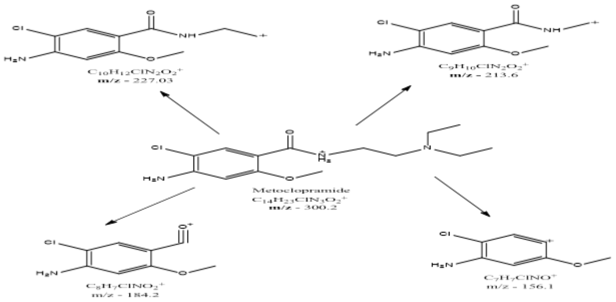

Figure 3: MCP fragmentation pathway

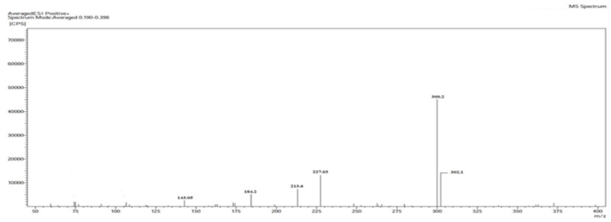

Figure 4: Line spectrum of MCP in LC-MS/MS study

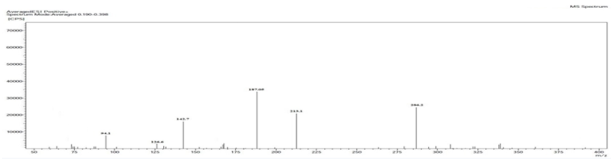

Figure 5: Line spectrum of DP-Iin LC-MS/MS study

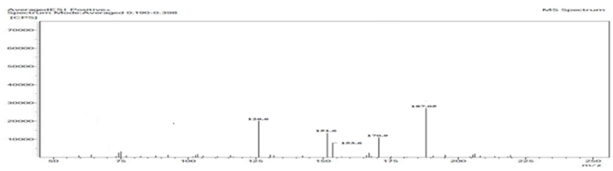

Figure 6: Line spectrum of DP-II in LC-MS/MS study

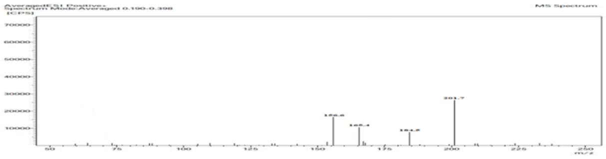

Figure 7: Line spectrum of DP-III in LC-MS/MS study

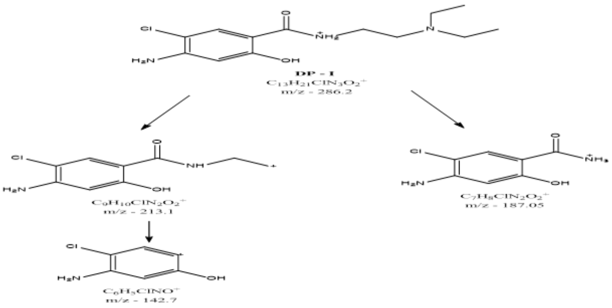

Figure 8: DP-I fragmentation pathway

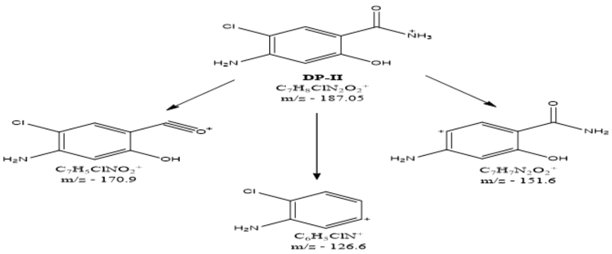

Figure 9: DP-II fragmentation pathway

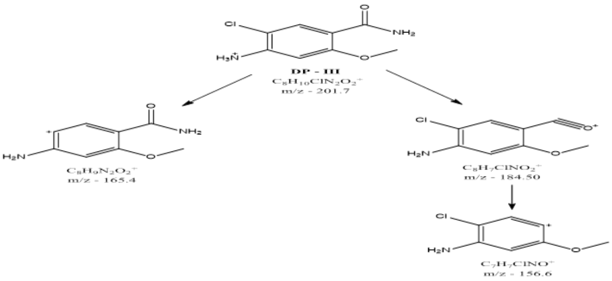

Figure 10: DP-III fragmentation pathway

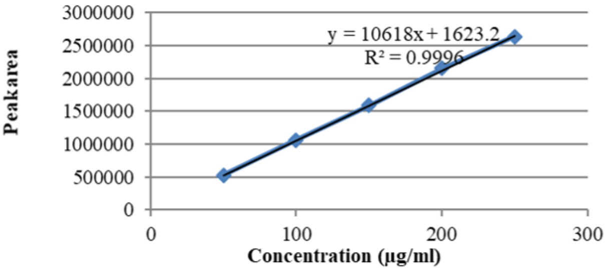

Figure 11: MCP linearity plot

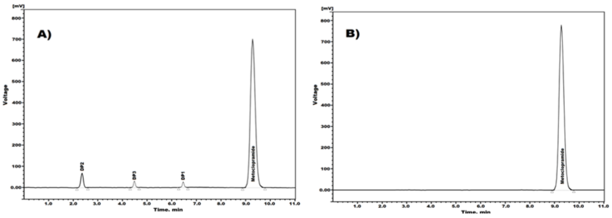

Figure 12: MCP representative chromatograms A) Acid degradation sample and B) Standard MCP solution

(m/z) of 300 produced fragmented product ions at m/z 227 resulted after the loss of $C_4H_{11}N$ (-73) that corresponds to the diethylamine side chain and another fragment at *m/z* 213 generated by the loss of $C_5H_{16}N_2$ (-116) indicating the cleavage of amide bond. Another one fragment ion appeared relatively in low abundance that has an *m/z* of 156 generated due to the loss of $C_7H_{16}N_2O$ (-144), indicating the complete cleavage of the side chain of metoclopramide from its phenyl ring system.

LC-MS/MS study of acid stressed sample

DP-I, II and III degradants mass spectrums were represented in Figures 5, 6 and 7. The possible molecular formula, experimental mass, major fragments and mass/charge ratio of all the DPs were shown in Table 1.

Identification of degradation compounds

All the DPs identification was processed with the help of fragment ions gained in LC-MS/MS study and compared with the fragmentation pattern of the analyte found in MS/MS analysis.

DP-I (m/z 286.78)

The electrospray ionization MS/MS spectrum of $[M + H]^+$ ions of DP1 (Rt = 6.47 min) shows molecular weight at m/z 286.2 with an elemental composition of $C_{13}H_{21}ClN_3O_2^+$ indicates the loss of $-CH_3$ from the metoclopramide from the methoxy group of phenyl ring by acid hydrolysis. Further, the fragmentation of DP1 produced fragment ions at m/z 213.1 and 187.05, 142.7, 126.6, and 94.1. The proposed scheme of fragmentation of DP1 was enumerated in Figure 8.

DP-II (m/z 187.05)

DP-II mass spectrum have shown molecular $[M + H]^+$ ion at (Rt = 2.36 min, Figure 12) m/z 187.05 with an elemental composition of $C_7H_8ClN_2O_2^+$ specifies the loss of diethylamine sidechain attached to the nitrogen of the amide group along with the $-CH_3$ from the metoclopramide from the methoxy group of phenyl ring by acid hydrolysis. The proposed fragmentation pattern of the DP-II was specified in Figure 9 as per the masses obtained in the MS/MS spectrum of DP-II. Fragmentation of DP-II produced peaks at m/z 170.9,153.6, 151.6 and 126.6.

DP-III (m/z 201.7)

DP-III mass spectrum have shown molecular $[M + H]^+$ ion at (Rt = 4.52 min, Figure 12) m/z 201.7 with an elemental composition of $C_8H_{10}ClN_2O_2^+$ specifies the loss of diethylamine sidechain attached to the nitrogen of the amide group from the metoclopramide by acid hydrolysis. The proposed fragmentation pattern of the DP-II was specified in Figure 10 as per the masses obtained in the MS/MS spectrum of DP-II. Fragmentation of DP-II produced peaks at m/z 184.5, 165.4 and 156.6.

Method Validation

The linearity of the technique was assessed over the drug concentration range of 50.0 μg/ml to 250.0 μg/ml and the results were represented in Table 2 and the graph was represented in Figure 11. The regression equation, slope and correlation coefficient values were found to be y = 10618x + 1623.2, 10618 and 0.9996, respectively.

The proposed HPLC method has specificity related to each peak respective to the peak purity data obtained by utilizing a PDA detector. The single point threshold, resolution and peak purity data of analyte were represented in Table 3 and the chromatograms shown in Figure 12.

Accuracy study was established at 80.0, 100.0 and 120.0 μg/ml concentration levels and the findings were disclosed that the method has more accuracy for the estimation of MCP. The percentage recovery findings were found in the range of 98.4% - 101.8% and the findings were shown in Table 4.

Limit of detection (LOD) and limit of quantification (LOQ) values were evaluated from the linearity graph and were found to be 5.23 μg/ml and 17.44 μg/ml. The precision was estimated by %RSD. The inter-day and intra-day precision data were shown in Table 5 and the findings were 0.05% and 0.03%, respectively. Robustness was processed by creating small, deliberate variations in the flow ($\pm$0.1ml/min) and composition ($\pm$2%) of mobile phase and % RSD was evaluated for chromatographic parameters such as theoretical plates, tailing factor with respect to the variations were tabulated. The findings were satisfactory and represented in Table 6. System suitability and stability of solution (at 2-8°C) were processed and %RSD values were calculated. All the findings were within limits and the values were tabulated in Table 7. The standard solution was stable for more than 24 h.

CONCLUSION

A simple, accurate and specific stability-indicating RP-HPLC technique for the analysis of metoclopramide in the presence of its acid degradation products was developed and degradants were identified by LC-MS/MS method. Three acid degradation products were formed for MCP in acid hydrolysis. The chromatographic separation was achieved on a Luna C18(2) 100A,250×4.6mm 5micron column using isocratic mobile phase system consisting of 0.1% formic acid in water -ACN in the composition of 20:80%V/V by adjusting the movable phase at 1 ml/min flow rate with wavelength detection at 273 nm. In acid hydrolytic condition, 13-14% of the drug undergoing degradation and in that major degradant was found to be DP3. All the DPs were identified and characterized by utilizing LC-MS/MS system in combination with accurate mass values. This work may be useful in further identification and characterization of different process-related impurities.

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