EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 10
68-74
Year: 2016

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS of DIFFERENT BRANDS of ANTIDOPAMINERGIC AGENTS

Anisa Elhamili[1]*, Khalifa El-aroud[1], Massud Anwair[1], Mohamed Siaan[2], Ali Ammar[3], Naiema Elfazaa[1], Enas Tantawy[1] and Adel Abushoffa[1]

[1]Department of Medicinal & Pharmaceutical Chemistry, University of Tripoli, P.O. Box 13394, Tripoli, Libya.
[2]Department of Industrial Pharmacy, Faculty of Pharmacy, University of Tripoli – Libya.
[3]Department of Biology, Faculty of Science and Arts, Al-Gabal Al-Gharby University – Libya.

***Corresponding Author: Anisa Elhamili**
Assistant Professor, Department of Medicinal & Pharmaceutical Chemistry, University of Tripoli, P.O. Box 13394, Tripoli, Libya.

Article Received on 08/08/2016 **Article Revised on 29/08/2016** **Article Accepted on 20/09/2016**

ABSTRACT
In the present study, the quality of different brands of antidopaminergic agents such as metoclopramide and domperidone tablets which are available in the Libyan markets was estimated. Also, to investigate whether these brands are corresponding to the specification of the international standard of British Pharmacopoeia (BP). These different brands were purchased from private pharmacy in Tripoli city and the assay methods of these brands conducted using Japanese HPLC system and LC-solution software for Chromatographic data. The purity of domperidone and metoclopramide has been identified to detect any related impurities such as synthetic precursor or degradation product which may lead to incompliance of these brands with international standard of BP. The questioner performed in this study indicated high percentage of Domperidone was dispensed compared to Metoclopramide. The obtained results based on triplicate runs or more indicated that the % content was within the recommended limit and all tested brands are safe for use except brand 2 Domperidone.

KEY WORDS: Metoclobramide/ Domperidone/ Assay/ Quality Control/ HPLC.

1 INTRODUCTION
Metoclopramide and domperidone are antidopaminergic agents, metoclopramide (MTH) antagonizes dopamine (DA) at the receptor site and it is a selective DA2 antagonist. It antagonizes effect of DA in the central nervous system (CNS), other organ systems and effect on modularly chemoreceptor trigger zone (CTZ). It is centrally acting anti emetic primarily used to treat nausea, vomiting especially that associated with migraine and severe headache and facilitate gastric emptying in patient with gastro paresis.[1, 2] It is also used for the management of gastrointestinal motility disorder and gastrointestinal reflux and prevention of cancer chemotherapy. On 24 October 2013, the European Medicines Agency's Committee on medical products for human use recommended the changes of metoclopramide containing medicine due to the potential risk of serious neurological side effect (extra pyramidal).[1] Extra pyramidal effect (reported incidence is approximately 0.2% and 25% in aged and young patient, respectively) are well recognized adverse effects of drug with dopamine receptor antagonist properties.[2] Chronic use of metoclopramide has been linked with dyskinesia, thus, it is recommended that treatment not exceed 5 days.[1, 3] Additionally, metoclopramide therapy induced restlessness, bowel disturbance, dizziness and faintness after oral or parenteral administration. Constipation, hyperprolactinaemia, dysponea, anxiety, confusion and tremor have also reported.[1] In term of structure, metoclopramide is a derivative of benzamide (Fig 1A), metabolism of metoclopramide yield the major metabolite (4-amino-5-chloro-N-[2-(ethyl amino)ethyl]-2-methoxy benzamide), a second minor metabolite (4-amino-5- chloro-N-[2-(diethylamino) ethyl]-hydroxbenzamide) and other reported metabolites: N4-acetyl metoclopramide, metoclopramide-N4-sulphonate and 4-acetamido-5-chloro-2-methoxybenzoic acid. It is available in the form of tablet, intravenous and intramuscular injection.[1, 3] Domperidone on the other hand, is peripheral dopamine antagonist, it is used as an antiemetic and to control gastrointestinal (dyspepsia, gastro esophageal reflux, nausea and vomiting).[2, 4] It is a dopamine DA2 receptor antagonist used throughout the world due to its unique pharmaceutical activity; it provides relief from nausea by blocking receptors at CTZ. Also, to prevent the common side effect of most Parkinsonism medication, usually co-administrated with other dopaminergic antagonist such as apomorphine. Domperidone is not recommended in pregnancy, it is effective in all vomiting except motion sickness. It does not cross the blood brain barrier (BBB) and so it is suitable alternative to metoclopramide in young

people.[2, 5] In March 2012, health endorsed an advisory statement, published by Teva Canada Limited, indicating that health practitioners should exercise caution when prescribing domperidone at doses greater than 30 mg/day.[4] Most studies have looked at doses of 30 to 60 mg/day that most likely to cause side effect like for instance dry mouth, transient skin rash or itching, headache, thirst, abdominal cramps, diarrhea, drowsiness and nervousness.[3- 5] In terms of structure, domperidone is a derivative of benzimidazole (Fig 1B). It is available in tablet, suppositories, suspension and IV form. Metabolism of domperidone yeild 2,3-dihydro-2-oxo-1H-benzimidazole-1-propionic acid, 5chloro-4-piperidinyl-1,3-dihyro-benzimidazol-2-one and 5-hydroxydomperidone.[3] Different analytical techniques for assessment the quality of metoclopramide and domperidone have been proposed.[6-15] Thin layer chromatography and high performance liquid chromatography has widely been used for estimation of metoclopramide and domperidone either in pharmaceutical preparation or biological material.[7-10, 12, 14]. The aim of the present study was to estimate the quality of different brands of antidopaminergic Agents; metoclopramide and domperidone tablets available in the Libyan markets.

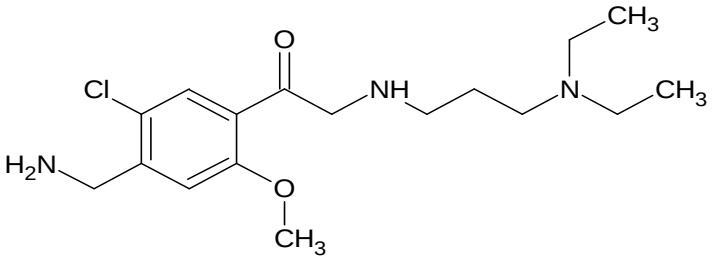

Fig 1A. Structure of Metoclopramide ($C_{14}H_{22}CLN_3O_2$, Mwt 299.80 g / moL).

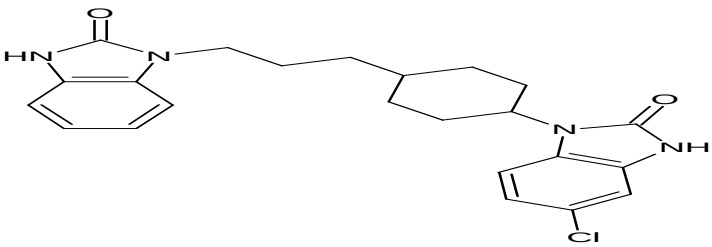

Fig 1B. Structure of Domperidone, ($C_{22}H_{24}CLN_5O_2$, Mwt 425.911 g/moL).

2 MATERIALS AND METHODS

Analysis is necessary for positive identification of drug purity. The purity of metoclopramide and domperidone need to be analyzed to detect any related impurities such as synthetic precursor or degradation product. The assay methods in this study were taken from the BP.[3, 12]

2.1 Metoclopramide

The method of assay was done according to the B.P.

Chemical materials

All the chemical materials and reagents used were HPLC grade. Two different brands of drug sample (metoclopramide tablet) was purchased from private pharmacy in Tripoli city, standard formulation RTC (Metoclopramide hydrochloride) lot NO: P500123 was provided by sigma-Aldrich MbH D-30918 Seelze, purity of STD 99.9%. Water, methanol, sodium acetate, sodium-1-octane sulphonate mono- hydrate for ion pair chromatography 99.0 % (T), filter membrane (0.45µl), syringe filter. HPLC grade glacial acetic acid.

Instrumentation

HPLC system made in JAPAN (SHTMADZU, model SPD-20AV) with LC-20AP pump & LC-20 AP UV detector was used. Chromatographic data was acquired using LC-solution software.

Chromatographic condition

The reversed-phase a stainless steel X-bridge C8 column 150 × 4.6 mm, particle size 5µm, column identification Lot No: 0111231511 were used as stationary phase. The buffer used was sodium-1-octane sulphonate monohydrate and sodium acetate-3- hydrate, pH was adjusted to 3.8 with HPLC grade glacial acetic acid. The buffer and methanol in ratio of (20:80 v:v) was used as mobile phase with flow rate of 1mL/min, the injection volume was 5 µl. Detection was carried out using UV detector at 305 nm at room temperature.

Buffer preparation

A mixture of 2.25 g of sodium-1-octane sulphonate monohydrate and 0.3 g of sodium acetate-3- hydrate was dissolved in sufficient water to produced 1000 mL. Filtration was done using membrane filter (0.45µm) and the pH was adjusted, if necessary, to 3.8 with HPLC grade glacial acetic acid, followed by degassing by ultrasonic bath. 200 mL of the buffer was taken and completed by methanol to 1000 mL.

Preparation of metoclopramide standard solutions
Standard stock solutions were prepared by transferring 25 mg of standard to 50 mL volumetric flask and 25 mL water was added, shaked mechanically for 20 min followed by ultrasoundic bath for 10 min to dissolve the content and then marked up with water. 5 mL of standard solution was taken and diluted to 50 ml with water, so as to get the concentration 0.05 mg/mL and then filtered through filter paper (Fig 2 A and B).

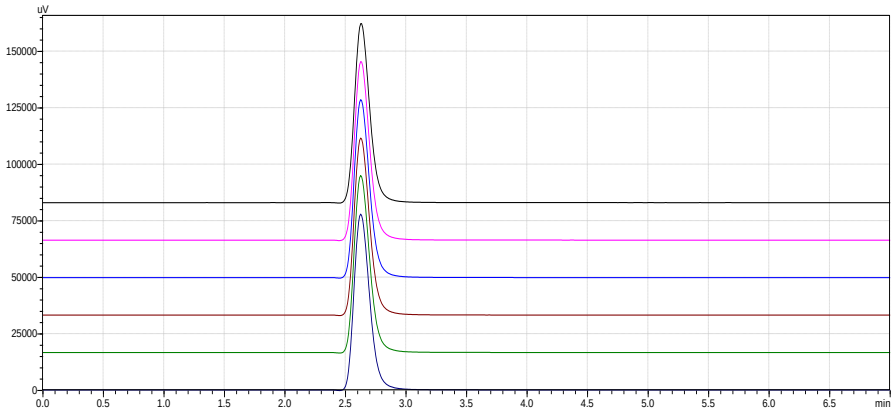

Fig 2A. Chromatogram of standard preparation 1 (Metoclopramide hydrochloride).

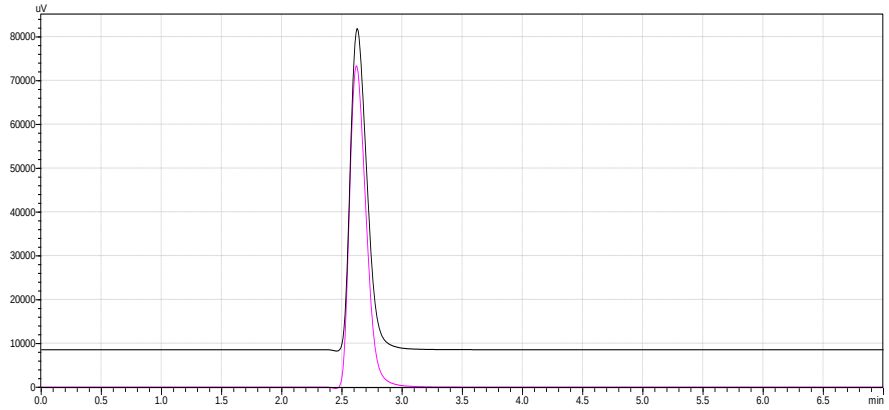

Fig 2B. Chromatogram of standard preparation 2 (Metoclopramide hydrochloride)

Preparation of sample solution
Sample solutions containing the drugs were prepared by dissolving tablets powder in water. 20 tablets of metoclopramide brand 1 and 2 were weighed separately and their average weights were determined. Powder of tablets equivalent to 10 mg of metoclopramide were weighed and placed in 100 mL volumetric flask. 55 mL of water was then added and dissolved by shaking mechanically for 2 min followed by ultrasonic bath for 2 min and marked up with the water. Then 10 mL was diluted in 100 mL volumetric flask and complete with water as to get the concentration of 0.01 mg / mL, then filtered using membrane filter.

2.2 Domperidone
The assay method was done according to the chapter 5-Shodhganga.[12]

Chemical materials
All chemicals used were HPLC grade. Two different brands of drug sample (domperidone tablet) were purchased from private pharmacy and standard formulation was provided by India CARETH (DOMPERIDIONE) in-house working standard DOM/ 10001013 purity 99.94%. Water, acetonitrile, orthophosphoric acid, potassium dihydrogen orthophosphate mol wt 136.00 of batch No 8701-17, syringe filter (0.45µm).

Instrumentation
HPLC system made in JAPAN "SHTMADZU" model SPD-20AV with LC-20 AP pump and LC-20AP UV detector was used. Chromatographic data was acquired using LC-solution software.

Chromatographic condition
Reversed- phase X select CSH C18 column (150×4.6 mm), particle size 5 µm was used as stationary phase. The buffer used was potassium dihydrogen phosphate; pH was adjusted to 3.0 with HPLC grade orthophosphoric acid. The buffer and diluents was in ratio of (50:50 v:v) and was used as mobile phase with flow rate 1 mL /min in isocratic programming, the injection volume was 20 µL, detection was carried out using UV detector at 272nm at room temperature.

Mobile phase preparation: The mobile phase consist of diluents (acetonitrile: water in ratio 50:50 v:v) and potassium dihydrogen phosphate buffer pH 3 filtered through filter membrane and degassed by ultrasonic bath.

Preparation of Domperidone standard solutions
Standard stock solutions were prepared by transferring 10 mg in 50 ml volumetric flask and 27 mL diluents was added and then sonicated with the aid of ultrasound for 2 min to dissolve the content then marked up with diluent, then diluted for stock standard solution 5 mL to 50 mL volume flask make up with diluent, so as to get the concentration 0.02 mg / mL, then filtered through filter paper (Fig 3 A and B).

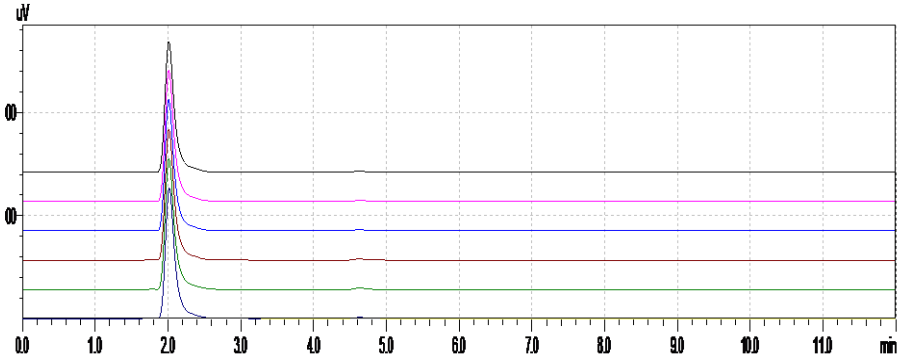

Fig 3A. Chromatogram of standard preparation 1 (domperidone)

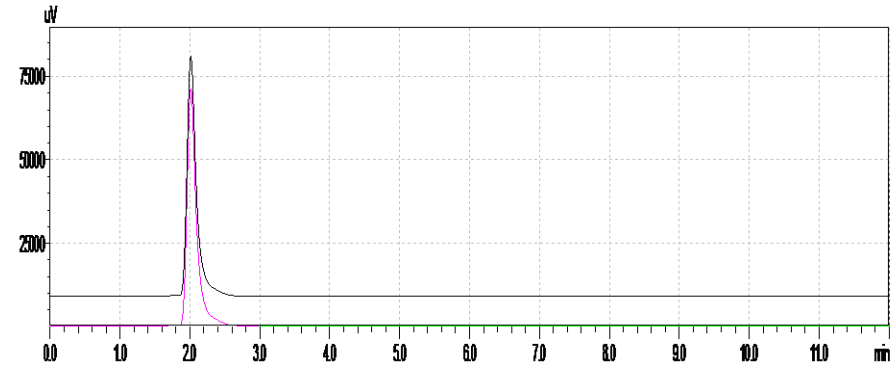

Fig 3B. Chromatogram of standard preparation 2 (domperidone).

Preparation of sample: Sample solution containing the drugs was prepared by dissolving tablets powder in to diluent. 20 tablets (domperidone brand 1&2) were weighed separately, their average weights were determined. Powder of tablets equivalent to 10 mg of domperidone were weighed and placed in 50 mL volumetric flask and dissolved by ultrasound for 2 min, then make up with the diluent. The diluted 5 mL in 50 mL volumetric flask make up with diluent so as to get the concentration 0.02 mg/mL, filtered through filter paper.

3 RESULTS AND DISCUSSIONS

Metoclopramide and Domperidone is antidopaminergic drug, Domperidone is safer than metoclopramide and it is less lipid soluble and low protein binding as compared with metoclopramide and they both differ in structure and mode of action. According to the questioner performed in this study, high percentage of domperidone was dispensed in private pharmacy compared to metoclopramide because of it is safety. Analysis of metoclopramide and domperidone by HPLC method is simple, precise and accurate. The analysis of both drugs were obtained in less than 5 min with good repeatability of migration time as it can be seen in Fig 4A and B for metoclobramide and Fig 5 A and B for domperidone. The %RSD of peak area for metoclopramide and domperidone standard was 0.18% and 0.02% respectively. Interestingly, the chromatogram of both tested drugs and their standards was also obtained in less than 5 min as it can be seen in Fig 2 and 3 with RSD value of 0.27% based on triplicate runs. The % assay of metoclopramide brand 1 and 2 was 101.6 % and 104.4 % respectively which is within the recommended BP range (90-110%). Additionally, retention time of both brands is almost the same (2.62 min). On contrast, the % assay of domperidone brand 1 and 2 was 101.97% and 90.77% respectively as it can be seen in Table 1 and 2. The BP limit is (95-105%) that means domperidone brand 1 is accepted and within the range whereas domperidone brand 2 not comply with the BP limit, this could be due to bad storage or transporting. Interestingly, the retention time of standard and sample is almost the same (2.01 min).

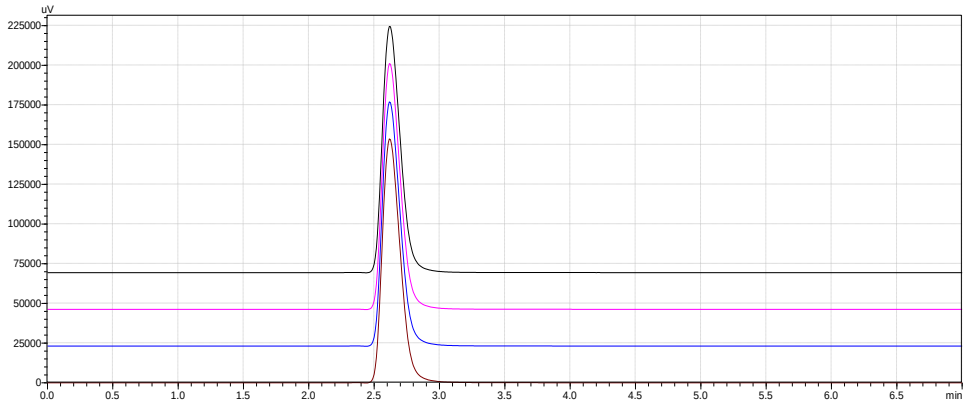

Fig 4A. Chromatograms of Metoclopramide brand 1 solutions prepared from commercial tablets.

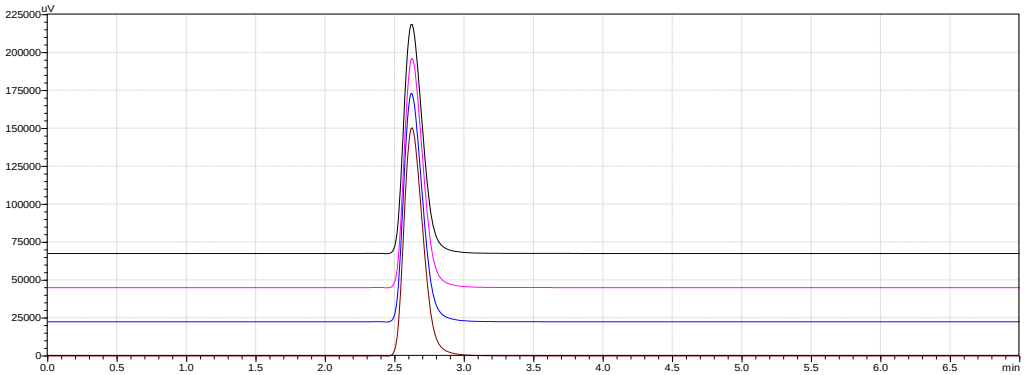

Fig 4B. Chromatograms of Metoclopramide brand 2 solutions prepared from commercial tablets.

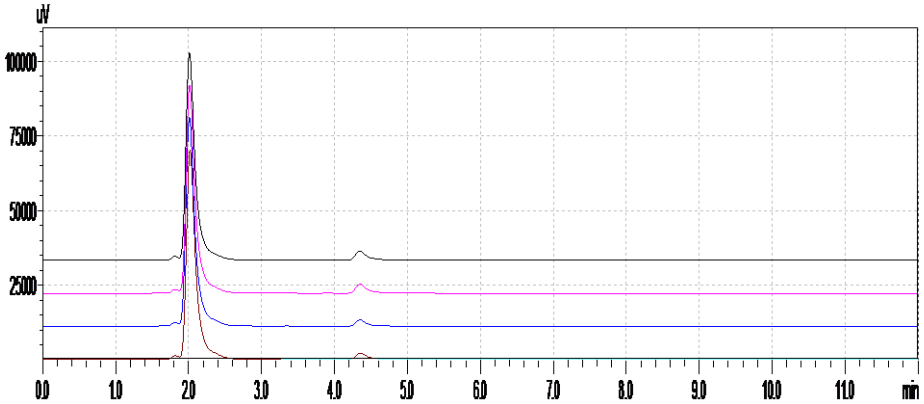

Fig 5A. Chromatograms of Domperidone brand 1 solutions prepared from commercial tablets.

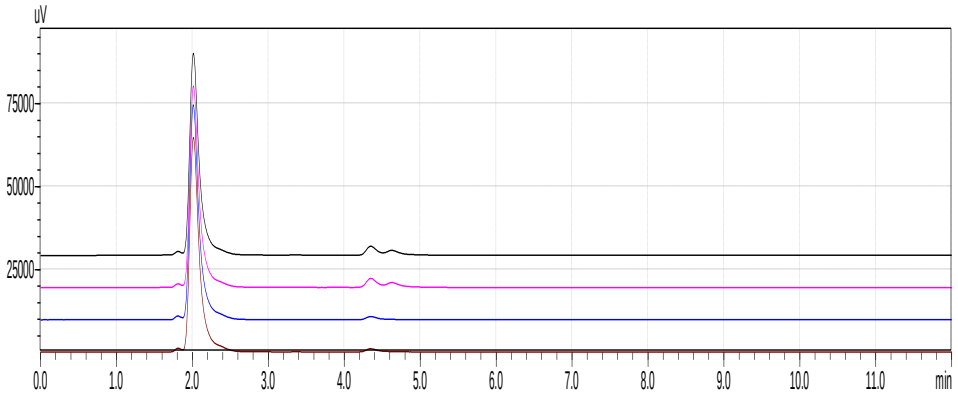

Fig5B. Chromatograms of Domperidone brand 2 solutions prepared from commercial tablets.

Table 1A: The obtained results of metoclopramide brand 1 by HPLC system.

No	Standard 1 metoclopramide 0.05 mg/ml	Sample A 0.1 mg/ml	Sample B 0.1 mg/ml	Standard 2 metoclopramide 0.05 mg/ml
1	703166	1436313	1434835	706142
2	704782	1442994	1434585	7056611
3	705107			
4	705968			
5	706632			
6	706358			
Average area	705336	1439654	1434710	705902
Average retention time	2.62	2.62	2.62	2.62
STD	1279.98			
RSD	0.1815%			
Average area	705619			705619
Weight	25	131	131.04	25
Concentration	0.05	0.1001	0.1001	0.05
Average conc	0.05	0.1001	0.1001	0.1001
Assay		101.79	101.41	
Average assay		101.60%	101.60%	

Table 1B: The obtained results of metoclopramide brand 2 by HPLC system.

No	Standard 1 metoclopramide 0.05 mg/ml	Sample C 0.1mg/ml	Sample D 0.09997 mg/ml	Standard 2 metoclopramide 0.05 mg/ml
1	703166	1480418	1468068	706142
2	704782	1478023	1476470	705661
3	705107			
4	705968			
5	706632			
6	706658			
Average area	705336	1479221	1472269	705902
Average retention time	2.62	2.62	2.62	2.62
STD	1279.98			
RSD	0.1815%			
Average area	705619			705619
Weight	25	126.92	126.42	
Concentration	0.05	0.1001	0.0997	0.05
Average conc	0.05	0.09999	0.09999	0.05
Assay		104.51	104.43	
Average assay		104.47%	104.47%	

Table 2A: The obtained results of domperidone brand 1 by HPLC system.

No	Standard 1 Domperidone 0.02 mg/ml	Sample A 0.02mg/ml	Sample B 0.02mg/ml	Standard 2 Domperidone 0.02mg/ml
1	603023	630924	638336	673829
2	603243	631039	639039	674013
3	602979			
4	602959			
5	603003			
6	602855			
Average area	603010	630982	673921	673921
Average retention time	2.01	2.01	2.01	2.01
STD	128.14			
RSD	0.0212%			
Average area	638466			638466
Weight	10.05	104.48	104.59	10.6
Concentration	0.0201	0.0200	0.02006	0.0212
Average conc	0.0206	0.0200	0.0200	0.0206
Assay		101.40%	102.53%	
Average assay		101.97%	101.97%	

Table 2B: The obtained results of domperidone brand 2 by HPLC system.

No	Standard 1 Domperidone 0.02 mg/ml	Sample C 0.02mg/ml	Sample D 0.02mg/ml	Standard 2 Domperidone 0.02mg/ml
1	603023	548422	584827	673829
2	603243	547700	584535	674013
3	602979			
4	602959			
5	603003			
6	602855			
Average area	603010	584061	584681	673921
Average retention time	2.01	2.01	2.01	2.01
STD	128.14			
RSD	0.0212%			
Average area	638466			638466
Weight	10.05	102.86	102.85	10.6
Concentration	0.0201	0.0200	0.02005	0.0212
Average conc	0.0206	0.0200	0.0200	0.0206
Assay		78.83%	93.77%	
Average assay		90.77%	90.77%	

4 CONCLUSIONS

The obtained results of the questioner during the two months, November and December 2015 indicated that most private pharmacy in Tripoli city dispensed domperidone about three time metoclopramide. The % assay of metoclopramide brand 1 and 2 was (101.60 %) & (104. 47 %) within the BP limit with the % RSD 0.18% and the retention time is the same for both samples and standard (2.62 min) with symmetrical peak shape. Whereas the % assay of domperidone brand 1 was 101.97% which is within the limit, however, the % assay of domperidone brand 2 was 90.71% which is below the limit rang. The % RSD 0.02% and the retention time was the same to the both sample and standard (2.01 min) with symmetrical peak.

5 ACKNOWLEDGMENTS

The authors would like to thank Faculty of Pharmacy, University of Tripoli for the support.

6 REFERENCES

1. ALBIBI, M. D., Mc., R. W., Ann Intern Med 1983; 98(1): 86-95.
2. David, P., WALDMANN, C., CHANDY, V., SHORT, S., et al., Br. J. clin. Pharmac. 1985; 19: 307-310.
3. B.P, 2008, 3, 2770.
4. Chun-Hui, Z., Bing-Xiang, Z., Yue, H., Ying, W., et al., Journal of Drug Delivery 2011; 1-9.
5. UKMI, TRENT MEDICINES INFORMATION SERVICE, RAPID COMMUNICATION 2014; 356-360.
6. David, P., Thomas, A., Robin, R., Kenneth, K., Jeffrey, B., THE AMERICAN JOURNAL OF GASTROENTEROLOGY 1999; 94: 1230-1234.
7. Ezzeldin, E., El-Nahhas, T., Tropical Journal of Pharmaceutical Research 2012; 11: 799-805.
8. Hesham, S., Safaa, R., Mamdouh, R., Kholoud, A., Pharmaceutica Analytica Acta 2012; 3: 1-4.
9. Kalirajan, R., Anandarajagopala, K., Seena, M., Gowramma, B., et al., Rasayan J. Chem 2008; 1: 232-235.
10. Kandimalla, S., Vasantha, K., WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES 2014; 356-360.
11. PRATIBHA, V., VARSHA, N., SONI, M., ASHOK, B., SUVARNA, B., International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5: 496-501.
12. Shodhganga(pdf), C.-. 356-360.
13. Siddappa, K., Mallikarjun, M., Reddy, P., Tambe, M., Ecl. Quím., São Paulo 2008; 33: 41-46.
14. Vamshikrishna, G., Neelima, M., Bhavani, M., Sreekanth, G., Shobha, R., IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 2012; 9: 69-78.
15. VASANTHARAJU, G., NAMITA, P., HUSSEN, S., International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4: 303-306.