Indian Journal of Biotechnology and Pharmaceutical Research 1 (1): 27-31 Sep 2013/Article ISSN 2347 - 3266

Simultaneous estimation for the analysis of Paracetamol and Domperidone in tablet formulations by RP-HPLC method

MEENAKSHI SINGH*, D. K. PATEL, OM SHANKAR, SAUMYA BAHADUR AND VIKAS GUPTA

Department of Analytical Chemistry of Indian Institute of Toxicology Research (CSIR), Lucknow, India

School of Pharmacy and Medical Sciences, Singhania University, Rajasthan, Distt. Jhunjunnu

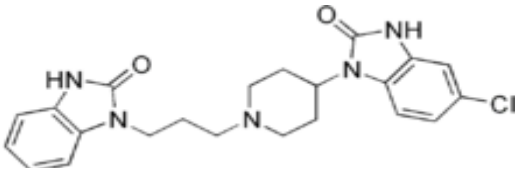
ABSTRACT

A simple, fast, precise and accurate reverse phase high pressure liquid chromatographic method was developed for simultaneous estimation of paracetamol and domperidone from tablet by reverse phase waters C_{18} column (250 x 4.6, mm, 5µm). The sample was analyzed using Sodium Phosphate Buffer (Na_2HPO_4 +NaH_2PO_4): Acetonitrile in the ratio of 60:40 v/v (pH adjusted to 7.5±0.1 with orthophosphoric acid) as mobile phase at a flow rate of 1.5ml/min & detection at 254 nm. The retention time for paracetamol & domperidone was found to be 2.1 & 8.5 min respectively. The validation of the proposed method was also carried out. The method was found to be linear (R^2 0.994 for paracetamol& R^2 0.997 for domperidone), precise (%RSD 0.98 for paracetamol & for domperidone 0.59) & selective. Due to its simplicity & accuracy, this method can be used for estimation of combination of these drugs in tablets dosage form.

Key words: Paracetamol, Domperidone, RP-HPLC

INTRODUTION

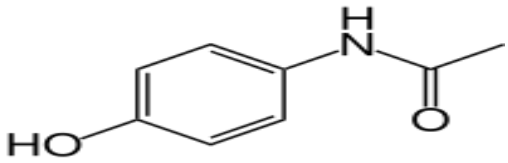
Domperidone - Domperidone (DOM) has the chemical name 5-chloro-1-[1-[3-(2, 3-dihydro-2-oxo-1H-benz-imidazol-1-yl) propyl]-4-piperidinyl]-1, 3-dihydro-2H-benzimidazol-2-one. It is a unique gastro kinetic and antiemetic drug. It is a peripheral dopamine -2-receptor antagonist, regulates motility of gastric and small intestinal smooth muscle and has been shown to have some effects on motor function of the oesophagus. It is generally used orally, rectally or intravenously to suppress nausea and vomiting (Ibrahim et al., 2011). There are several methods of HPLC have been developed for the estimation of domperidone individually (Zavitsanos et al., 2009; Kobylinska and Kobylinska, 2000).



Paracetamol - Paracetamol, chemically 4-hydroxy acetanilide, is a centrally & peripherally acting non-opioid analgesic and antipyretic. It is commonly used for the relief of fever, headaches & other minor aches & pains. This

*Corresponding author: **meenakshisingh980@gmail.com**

combination is used for antiemetic and pain associated with gastrointestinal disorders and also used for the treatment of migraine. The used of paracetamol also in the management of moderate to severe pain (Tripathi, 2005; Goyal, 2006). There are several methods of paracetamol have been analysed individually and combination of different drugs by different type of equipments such as HPLC, RP HPLC and UV (Karla et al., 2009; Sridhar et al., 2009; Wafaa, 2008; Karthik et al., 2007; Joshi and Sharma, 2008).



Karthik et al (2007) and Yadav et al (2009) estimated hybrid method of paracetamol and domperidone in tablets as a dosage form and formulation by different instrumental techniques such as reverse phase HPLC chromatography, Spectrophotometric and HPTLC (Kapil et al., 2009).

Indian and British Pharmacopoeias recommend UV spectrophotometric and titrimetric assay method for paracetamol in bulk and tablet production while in domperidone only titrimetric assay method of is official in British Pharmacopoeia (Indian Pharmacopoeia. 1996; British Pharmacopoeia. 2002).

MATERIAN AND METHODS

Instrumentation/Apparatus and analytical conditions - Chromatographic analysis was performed using HPLC system (Waters Miliford, MA, USA), which consists of Waters-515 binary pump, Rheodyne injector-7725 with 20 µL loop, waters column C-18 (250 x 4.6 mm, 5µm) maintained at ambient temp and Waters UV detector-2487 at 254 nm**.** Reversed-phase HPLC analyses were performed and separation was achieved with a mobile phase composition of Sodium phosphate buffer solution and Acetonitrile (60:40, vol/vol) isocratic solvent system. Sodium phosphate buffer solution was prepared using 20 mM each concentration of NaH_2PO_4 and Na_2HPO_4 with the ratio of 1:1, v/v. The mobile phase was prepared freshly before use and filtered through 0.45 µm membrane filter (Milli-pore) and degassed by ultra sonication bath before use.

Table-1 Optimized chromatographic conditions.

PARAMETERS	OPTIMIZED CONDITIONS
Chromatograph	Waters-HPLC
Column	Waters column C-18 (250 x 4.6, mm, 5µm)
Mobile phase	Buffer (Na_2HPO_4+NaH_2PO_4) and Acetonitrile (60 : 40, v/v)
Flow rate	1.5 ml/min
Detection	254 nm
Injection volume	20µl
Temperature	Ambient
Retention time –Paracetamol	2.1
Retention time -Domperidone	8.5

Materials and chemical - Pure sample of paracetamol and domperidone were obtained as gift samples from Vasudha Pharma Ltd. Hyderabad for estimation of paracetamol and domperidone in commercial formulations. HPLC grade orthophosphoric acid, Acetonitrile & Methanol were procured from Merck, India. High purity water was prepared by using Millipore milli Q plus purification system. The pharmaceutical dosage form used in this study was marketed formulation, labled to contain 10 mg domperidone & 500 mg paracetamol, which were obtained from the local market.

Preparation of mobile phase - Weighed accurately Na_2HPO_4 (1.420 gm) and NaH_2PO_4 (1.200gm), dissolved in 1000 ml volumetric flask, sonicated & filtered through vacuum filter. Adjusted the pH 7.5±0.1 with orthophosphoric acid through the pH meter & made mobile phase in the

ratio of 60:40 (20 mM buffer: ACN) & then sonicated for 30 min.

Preparation of standard stock solution - 10mg/10ml of domperidone & paracetamol were prepared by dissolving 10 mg of drug in 10 ml of methanol, separately. The solutions were suitably diluted with mobile phase to get mixed standard solution containg 1 µg/ml of paracetamol and 6 µg/ml of domperidone.

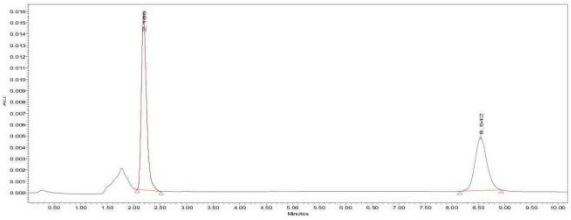
Preparation of sample solution - Ten tablets, each tablet was labeled containing 10 mg of domperidone & 500 mg of paracetamol, were weighed & powder equivalent to 10 mg of paracetamol was weighted accurately and taken into 10 ml volumetric flask. The drugs were extracted in to methanol; volume was adjusted to 10ml, vortexed & then filtered through 0.45 µ membrane filter. From this solution, further dilutions were made using mobile phase to get a final concentration of 1 µg/ml of paracetamol and 6 µg/ml of domperidone. (Fig.1) 20 µl of solution was injected into HPLC system to obtain chromatogram for standard drug solution (5-repicates) & sample solution (replicates).Concentration of paracetamol and domperidone in the formulation were calculated by comparing AUC of sample with that of standard.

RESULTS AND DISCUSSION

The present RP-HPLC method was validated as per ICH guidelines. In past work carried out on developed hybrid method of paracetamol and domperidone showed the set of wavelength 230 nm (Karthik et al 2007).but in present study wavelength was fixed at 254 nm and the Retention time of DOM is slightly more and less of PCM by the compare of reported method.

Chromatographic Method - Initially, Phosphate buffer and Acetonitrile were tried as mobile phase, but satisfactory peak was not found. Then Sodium Phosphate Buffer and Acetonitrile in the ratio of 60:40 was found to satisfactory with two well-resolved peaks for PCM and DOM. Optimized chromatographic conditions are listed in Table-1 and a representative graph is shown in Fig.1.

Fig. 1: Chromatogram for 1ppm paracetamol and 6ppm domperidone tablet



System Suitability - The system suitability was tested for checking the various parameters such as column efficiency, resolution, tailing factor, LOQ, LOD and theoretical plates. Peaks were well resolved with good resolution between the two drugs. The assay concentration of 1 µg/ml of paracetamol and 6 µg/ml of domperidone was selected. The asymmetry factors of all the peaks were lesser than 2.0 & it showed that all peaks were symmetrical in shape. The results obtained are shown in Table -2.

Table-2 System Suitability Parameters

PARAMETERS	PARACETAMOL	DOMPERIDONE
Calibration range	10-2 ppm	10-2 ppm
Theoretical plates	4430	6550
Tailing factors	1.76	1.19
LOD	0.007 ppm	0.1 ppm
LOQ	0.031 ppm	0.3 ppm

Accuracy and Precision - Accuracy of method was ascertained by recovery study done by spiking the already analysed sample of the tablet with their different known concentration of PCM and DOM. The mean% recovery was found to be (98.50%) for paracetamol and (98.32%) for domperidone. The precision of the proposed method was lesser than 2% for both drugs,

when it was injected 6 times and there was good repeatability of the proposed method. The results have been shown in Table-3.

Table-3 Recovery studies of tablet

Drugs	Amount added (µg/ml)	Amount recovered (µg/ml)	Recovery (%)	Average recovery (%)
PCM	6.251	6.110	97.74%	
	6.528	6.425	98.42%	98.50%
	6.812	6.759	99.22%	
DOM	2.252	2.195	97.46%	
	2.826	2.795	98.90%	98.32%
	2.321	2.289	98.62%	

Linearity - Linearity was observed in the concentration range of 10-2µg/ml for both drugs with the correlation coefficient of (R^2 0.999) for paracetamol & (R^2 0.999) for domperidone respectively & calibration curve was plotted using AUC versus concentration of standard solution. The other validation parameters are summarized in Table-4.

Table -4 Validation Parameters of tablets

PARAMETERS	PARACETAMOL	DOMPERIDONE
Recovery	98.50%	98.32%
Repeatability	0.43%	0.70%
Reproducibility	1.024%	1.22%
Precision	0.98	0.59
Linearity	0.999	0.999

Specficity - Specificity was carried out by exposing the sample to different stress conditions for 10 hrs such as acidic (0.1N HCL, 1ml 40°C); basic (0.1N NaOH, 1ml 40°C); heat (1ml, 60°C); sunlight (1ml, 40° C), before analysis, by proposed method. The results of specificity studies indicated no interferences from excipients, impurities, and degradation products under various stress conditions and assured that the peak response was due to single component only.

The proposed HPLC method was simple & precise because of the commonly used buffer & shorter runtime. The proposed method is highly accurate which showed good recovery of the drug sample & it is cost effective, faster and can be used for the routine analysis of these drugs from tablet formulations.

ACKNOWLEDGMENTS

The authors are thankful to Indian Institute of Toxicology Research, (CSIR), Lucknow and Singhania University, Rajasthan, India, for providing the facilities to carry the experiment.

REFERENCES

British Pharmacopoeia. 2002. Her Majesty Stationery Office, London, 1.

Goyal RK. 2006. Elements of pharmacology. 16th Edition, B.S.Shah Prakashan. 171- 182.

Ibrahim EH, El-Faham TH, Mohammed FA and El-Eraky NS. 2011. Enhancement of solubility and dissolution rate of domperidone by utilizing different techniques. Bull. Pharm. Sci., Assiut University, **34**: 105-120.

ICH, Validation of Analytical procedure: Methodology (Q2B), International Conference on Harmonization, IFPMA, Geneva, 1996.

Indian Pharmacopoeia. 1996. Govt. of India, Ministry of Health and Family Welfare, Delhi. Publication by Controller of Publication, **2**.

Joshi R and Sharma R. 2008. Development and validation of RP-HPLC method for simultaneous estimation of three-component tablet formulation containing acetaminophen, chlorzoxazone and aceclofenac. *Analytical Letter* **41**: 3297.

Kapil K, Naik S, Garima J and Mishra N. 2009. Spectrophotometric method for simultaneous estimation of paracetamol and domperidone in tablet formulation. *Asian Journal of Research in Chemistry* **2**: 112-114.

Karla K, Naik S, Jurmal G and Mishra N. 2009. Spectrophotometric method for simultaneous estimation of paracetamol and domperidone in

tablet formulation. *Asian Journal of Research in Chemistry* **2:** 112

Karthik A, Subramanian G, Ranjith Kumar A and Udupa N. 2007. Simultaneous estimation of paracetamol and domperidone in tablets by reverse phase HPLC method. *Indian Journal of Pharmacy Science* **69**: 142-144.

Kobylinska M and Kobylinska K. 2000. High-performance liquid chromatographic analysis for the determination of domperidone in human plasma. *Journal of Chromatogr* **10**: 207.

Sridhar N, Pradeep K, Rakesh S, Ayushman T and Manik G. 2009. Simultaneous analysis of paracetamol and tramadol - analytical method development and validation. *Dermetology of Pharmacy Chemical* **1**: 72.

Tripathi KD. 2005. Essential of Medical Pharmacology. 6th Edition, Jaypee brothers, 259-266.

Wafaa SH. 2008. Determination of ibuprofen and paracetamol in binary mixture using chemometric-assisted spectrophotometric methods. American Journal of Applied Science. **5**: 1005-1008.

Yadav A, Raman MS, Satish CM, Pawan KS and Gyanendra NS. 2009. A simple and sensitive HPLC method for simultaneous analysis of domperidone and paracetamol in tablet dosage forms. *Journal of Planar Chromatography* **22**: 421-424.

Zavitsanos AP, MacDonald C, Bassoob E and Gopaul DN. 1999. Determination of domperidone in human serum and human breast milk by high-performance liquid chromatography-electrospray mass spectrometry. *Journal of Chromatogr B* **9**: 730.