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Development and Validation of RP-HPLC method for the Simultaneous Estimation of Paracetamol, Domperidone and Esomeprazole magnesium in Tablet Dosage Form

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Abstract : A simple, precise, accurate and rapid reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of paracetamol (PCM), domperidone (DOM) and esomeprazole magnesium (ESO) in tablet dosage form. The chromatographic separation was achieved on Sunfire C18 column (150mm × 4.6mm, 3.5 µ) as stationary phase with mobile phase consist 0.02 M phosphate buffer along with ortho- phosphoric acid adjusted to pH-3.0 and acetonitrile in the ratio of 70:30 v/v with a flow rate of 0.5 ml/min, UV detection at 277 nm. The retention times of paracetamol, domperidone, and esomeprazole magnesium were 4.1 min, 6.3 min and 9.5 min respectively. The linearity of paracetamol, domperidone, and esomeprazole magnesium were in the range of 10-100µg/ml with correlation co-efficient greater than 0.999. Assay recoveries for paracetamol, domperidone, and esomeprazole magnesium were found to be 99.92%, 100.03%, 99.40% respectively. The results of study show that the proposed RP-HPLC method was found to be simple, accurate, precise and rapid which can be success fully used for the determination of paracetamol, domperidone, and esomeprazole magnesium in pharmaceutical dosage forms.
Key words: HPLC, Paracetamol, Domperidone, Esomeprazole magnesium.

Introduction

Paracetamol (PCM) is chemically acetamide, N - (4-hydroxy phenyl) – 4 –hydroxy acetanilide, it is centrally and peripheripherally acting non-opioid analgesic and anti-pyretic. Paracetamol does not irritate the lining of stomach or affect blood coagulation. At normal therapeutic doses, it is metabolized very fast and completely by undergoing glucuronidation and sulphonation to inactive metabolies that are eliminated in the urine[1]. Domperidone (DOM) 5- chloro- 1-(1 –(3- (2,3 –dihydro -2- oxo -1H –benzimidazol -1 –yl) propyl) - 4 – piperidinyl) -1,3 –dihydro – 2H –benzimidazole -2 –one is a synthetic benzimidazole compound that acts as dopamine antagonist with antiemetic and suppress nausea and vomiting. It is also used as prokinetic agent for treatment of upper gastro intestinal motility disorders[6]. It can be used in patients with parkinson's disease and also found to be effective in the treatment of gastroparesis[7]. Esomeprazole (ESO) magnesium trihydrate is chemically bis (5 – methoxy – 3,5 – dimethyl -2 – pyridinyl) methyl) sulfinyl) – 1H – benzimidazol – 1- yl) a compound, it is a proton pump inhibitor that suppresses gastric acid secretion. Esomeprazole is cost effective in the treatment of gastric oesophageal reflux diseases[7]. The S – and R – isomers of omeprazole are protonated and converted in the acidic compartment of the parietal cell forming the active inhibitor, the achiral sulphenamides[6].. Literature review reveals that method has been reported for analysis of PCM, DOM and ESO either alone or in combination with other drugs. The reported methods are HPLC[1-8,18-22], stability indicating HPLC[16], bioanalytical[15] , Spectrophotometric[10-12], HPTLC[9]. Till date, there was no method reported for

simultaneous estimation of PCM, DOM and ESO by HPLC method in tablet dosage form. The proposed method is validated as per ICH guidelines.

Material and Methods

Chemicals and reagents:

HPLC grade methanol and acetonitrile were purchased from Sigma-Aldrich, India. The reference standard of paracetamol, domperidone and esomeprazole magnesium was purchased from Sigma-Aldrich, India. Sample products of P-500 manufactured by Apex pharmaceuticals Ltd, Chennai, with label claim of Paracetamol 500mg, Domstal manufactured by Torrent pharmaceuticals Ltd, Ahmedabad, with label claim of Domperidone 10mg and Esoz manufactured by Glenmark pharmaceuticals Ltd, Mumbai, India, with label claim of Esomeprazole 20mg was purchased from local market. Sodium dihydrogen phosphate and ortho - phosphoric acid HPLC grade were purchased from Himedia laboratories pvt Ltd, Mumbai, India. Ultra pure water was obtained from a mille Q water purification system from Milipore (Mlliford USA).

Apparatus and chromatographic conditions

The analysis was carried out on a Waters HPLC system equipped with UV detector, pressure controlled by prominence pump and operated by Empower-2 software. Sunfire C18 column (150mm × 4.6mm i.d, particle size 3.5µm) was used for separation. Mobile phase consist (0.02M) sodium dihydrogen phosphate buffer with o-phosphoric acid adjusted to pH-3.0 and acetonitrile in the ratio of 70:30 v/v. The flow rate was kept at 0.5 ml/min, eluents were detected by UV detector at 277nm.

Preparation of standard stock solution

Standard stock solution was prepared by weighing accurately 10mg each of paracetamol, domperidone, esomeprazole magnesium individually. Dissolve in methanol in 10ml volumetric flask and made up volume with methanol. From above stock solution 1ml each was taken separately and diluted with 10ml methanol to obtain a final concentration of 100µg/ml. Further dilutions from these solutions were made with mobile phase.

Sample preparation

10 tablets of each paracetamol, domperidone, esomeprazole magnesium was taken individually and weighed. The weighed tablets were powdered. A quantity of powder equivalent to 500mg of paracetamol was weighed and transferred into 50ml volumetric flask to get 10mg/ml with methanol. Further diluted with methanol to get 250µg/ml. A quantity of powder equivalent to 10mg of domperidone and 20mg of esomeprazole magnesium was weighed and transferred into 10ml volumetric flask to get 1mg/ml with methanol. Further diluted with methanol to get 250µg/ml.

Results

Method development

In the present work, conditions were optimized for the development and validation of a simple and accurate HPLC method for the simultaneous estimation of PCM, DOM and ESO in tablet dosage form. The changes in the retention time of all drugs were noted as function of changing mobile phase concentration, pH, flow rate and column. Initially Acetonitrile: 0.05 M phosphate buffer (pH 5) in the ratio of 60:40 v/v was performed. The retention time was found to be PCM-2.9 min, DOM- 2.3 min and ESO- 3.9 min respectively. The peaks of PCM and DOM are not well resolved. Ratio of mobile phase changed to 50:50 v/v Acetonitrile: 0.05 M phosphate buffer (pH 4 adjusted with ortho phosphoric acid). The retention time was found to be PCM-3 min, DOM- 3 min and ESO- 4.4 min respectively. But two drug peaks were eluted at the same retention time (RT-3 min). Again changed the ratio of mobile phase 40:60 v/v of Acetonitrile: 0.02 M phosphate buffer along with pH 3 adjusted with ortho phosphoric acid was used. The retention time was found to be PCM- 3.7 min, DOM- 3.1 min and ESO- 5 min respectively. But separation was not proper. Hence the column of Phenomenex luna C18 (150mm × 4.5mm, 5µ) was changed with Sunfire C18 (150mm × 4.5mm, 3.5µ) to get well resolved peaks. Finally Acetonitrile: 0.02 M sodium dihydrogen phosphate buffer (pH 3.0 adjusted with ortho

phosphoric acid) in the ratio of 70:30 % v/v at flow rate 0.5 ml/min gave acceptable retention time and good resolution between PCM, DOM and ESO respectively.

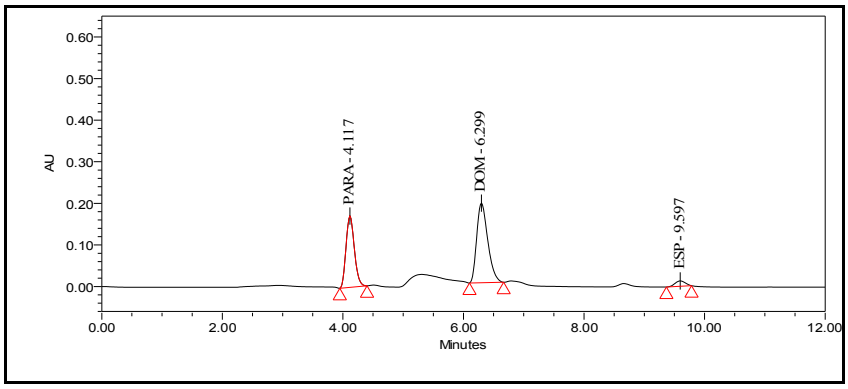

Figure 1: Chromatogram for standard PCM, DOM and ESO

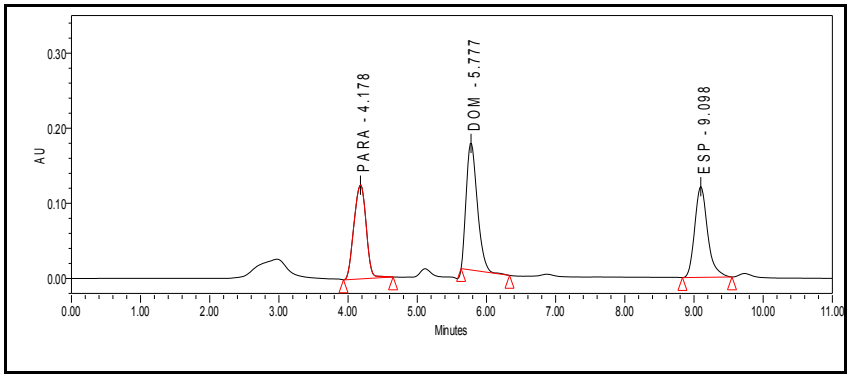

Figure 2: Chromatogram for sample on simultaneous estimation of PCM, DOM and ESO

Method validation

The proposed method was validated by studying several parameters such as specificity, linearity, precision, accuracy and limit of detection (LOD), limit of quantification (LOQ) and system suitability.

Specificity

The specificity of the method was checked by peak purity test of the sample solution by UV detector. Peak purity for PCM, DOM, ESO are observed to be 0.9999, 0.9997, 0.9994 respectively. The results of peak purity analysis shows that the peak of analytes was pure and excipients in the formulation are not interfering with the analyte peaks.

Linearity

The linearity of the method was established at eight concentration levels ranging from 10-100 µg/ml (10, 20, 30, 40, 50, 60, 80, 100µg/ml) for PCM, DOM, and ESO respectively. The calibration curves were plotted between response factor and concentration of the standard solutions. The linearity ranges were found to be 10-100 µg/ml with correlation coefficient of 0.999 (r^2) for PCM, DOM and ESO respectively.

Limit on Detection (LOD) and Limit on Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were established by injecting the low concentrations of the standard solution using the developed RP-HPLC method. The LOQ is the smallest concentration of analyte that gives measurable response. The method was found to be within the limit. The results were shown in Table 1

Table-1: LOD and LOQ

Method	Range (µg/ml)	R^2	LOD (µg/ml)	LOQ (µg/ml)
RP-HPLC-PCM	10 – 100 µg/ml	0.9999	0.17	0.50
RP-HPLC-DOM	10 – 100 µg/ml	0.9997	0.10	0.32
RP-HPLC-ESO	10 – 100 µg/ml	0.9994	1.03	2.62

Accuracy

Accuracy was done by the standard addition method. The recovery and relative standard deviation for each of the analytes is given. Three levels of solution were taken at nominal analytical concentrations such as 25, 50 and 75% respectively. From recovery studies, the method was reliable and accurate. The results are shown in Table 2.

Table-2: Recoveries data for PCM, DOM and ESO

DRUGS	Level %	Amount added (conc.)µg/ml	Amount found µg/ml	% Recovery ±SD	% RSD
PCM	25	15	14.96	99.77 ±1.38	1.3
	50	20	20.10	100.5 ±0.98	0.9
	75	25	24.97	99.88 ±0.96	0.9
DOM	25	25	24.89	99.60 ±1.42	1.4
	50	30	29.95	99.83 ±1.35	1.3
	75	35	34.97	99.92 ±0.83	0.8
ESO	25	30	29.70	99.02 ±1.56	1.5
	50	40	40.02	100.05 ±0.44	0.4
	75	50	49.86	99.72 ±0.91	0.9

*Mean of three determinations, RSD-Relative standard deviation

Table-3: Precision for intra day

DRUGS	Concentration	Mean	Standard deviation	% RSD
PCM	25.02	3	0.11	0.4
	50.20	3	0.09	0.1
	75.10	3	0.57	0.7
DOM	25.12	3	0.17	0.6
	50.35	3	0.09	0.1
	75.01	3	0.37	0.4
ESO	25.03	3	0.14	0.5
	50.30	3	0.19	0.3
	75.25	3	0.11	0.1

Table-4: Precision for inter day

DRUGS	Concentration	Mean	Standard deviation	% RSD
PCM	25.02	3	0.22	0.8
	50.20	3	0.28	0.5
	75.10	3	0.56	0.2
DOM	25.12	3	0.15	0.6
	50.35	3	0.28	0.5
	75.01	3	0.55	0.7
ESO	25.03	3	0.17	0.7
	50.30	3	0.18	0.3
	75.25	3	0.32	0.4

Precision

The precision of the method was assessed as repeatability and intermediate precision by preparing three different solutions at low, medium and high concentrations, which were freshly prepared and analyzed six times on the day. These experiments were reported over a three days period to evaluate day-to-day variability. The method precision is satisfactory and the % RSD is not more than 2.0%. Results are given in Table 3 and 4

Robustness

Robustness of the method was performed by small deliberate changes in the optimized chromatographic conditions such as mobile phase composition (5 %) and pH of Phosphate buffer (±0.1units). Then the standard deviation of the peak area was calculated for each change of conditions % RSD was found to be less than 2. It indicated the method is robust.

System suitability

System suitability was studied under validation parameters by injecting six replicate injections of the standard solution. System suitability parameters like column efficiency, plate count, tailing factor were recorded. The results obtained were within the limit.

Assay

The assay repeated for three times and the peak areas were recorded and the amount of drug formulation of PCM, DOM and ESO was calculated using a standard calibration curve by taking concentration in µg/ml. The results of the assay are shown in Table 4.

Table 4: Assay recoveries of PCM, DOM and ESO

Drugs	Label claim (mg)	Drug content ± SD	% RSD
PCM	500	99.93 ± 0.82	0.82
DOM	10	100.04 ± 0.93	0.93
ESO	20	99.39 ± 1.02	1.02

Discussion

The developed RP-HPLC method was found suitable for simultaneous estimation of PCM, DOM and ESO with good resolution, peak shapes and less tailing. The correlation coefficient was 0.999 showed the stable, linear detector response in different concentration of PCM, DOM and ESO respectively. The proposed method was validated as per ICH guidelines. The percent recoveries for PCM, DOM and ESO were 99.2%, 100% and 99.4% respectively indicating the accuracy of the proposed method. LOD and LOQ values indicate good sensitivity of proposed method. The % RSD values was less than 2 for intra and inter day variation studies indicated that proposed method was precise. The developed method was studied for percentage recovery at three concentration levels and % RSD values of less than 2 in variation in pH and mobile phase ratio indicate that method was robust. The sample recoveries of PCM, DOM and ESO from commercial tablet formulation were agreed well with their label claim indicating that there was no interference from the commonly used tablets excipients.

Conclusion

A simple, precise, reliable, rapid, sensitive and accurate reverse phase HPLC method has been developed for the simultaneous estimation of paracetamol, domperidone and esomeprazole magnesium. The developed method is suitable for the identification and quantification of two different combinations as well as individuals of paracetamol, domperidone and esomeprazole. The analytical conditions developed with good resolution within a short analysis time. % RSD for all parameters was found to be within the limit.

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