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Simultaneous Estimation of Sumatriptan Succinate, Metoclopramide Hydrochloride and Paracetamol by RP-HPLC Method

Aaditya Singh, Shiv Bhadra Singh,

Faculty of Pharmacy, Aryakul college of Pharmacy and Research Lucknow, India

Abstract

The aim of the study was to develop and validate Reverse Phase High Performance Liquid Chromatography (RP-HPLC) assay for simultaneous estimation of 5-HT agonist, antiemetic and analgesic drugs (Sumatriptan succinate, Metoclopramide HCl and Paracetamol). Chromatographic separation achieved isocratically on a C_{18} column, utilizing a mobile phase of KH_2PO_4 buffer:MeOH (60:40 v/v), pH 5 at a flow rate of 0.5 ml/min with a UV detection at 230 nm for 6.8 min, 248 nm from 6.81 to 8.5 min and 213 nm for rest of the time using Shimadzu Chromatographic HPLC System and Hamilton, Bondaz AZ microliter syringe. Sample solutions were prepared in the mobile phase with concentration range of 0.5-32 µg/mL by the serial dilution technique. Chromatogram was obtained in which the retention time were 6.1, 7.4 and 10.8 min for sumatriptan succinate, paracetamol and metoclopramide HCl respectively. The linearity of sumatriptan succinate, paracetamol and metoclopramide HCl was in range of 0.5-32 µg/mL. The coefficient of correlation (r^2) for sumatriptan succinate, paracetamol and metoclopramide HCl were found to be 1, 0.999 and 0.999 respectively over a range of 0.5-32 µg/mL.

This method was validated by determining its accuracy, precision, linearity, reproducibility and specificity according to ICH guidelines and used for quality control and routine bulk drug analysis.

INTRODUCTION

Headache is the most common complaint of patients seen by the physician. It may be serious symptoms of intracranial structural changes caused by brain tumors, cerebrovascular disorders like aneurysms and angiomas. Other organic disorders causing headache include glaucoma, hypertension and chronic sinusitis. The nerve cells of the brain itself are not sensitive to the pain- producing stimuli, but arteries causing the membrane covering the brain contain pain-sensitive nerve –endings. When these intracranial arteries are distended, pain i.e. perceived. Nerve impulses also arise from extra cranial structures like skin, blood vessels and muscles of the scalp and neck, which becomes a source pain. In addition, psychic causes may also cause a kind of chronic headache.

Thus headache may be divided into two:

- Tensions, or muscle contractions headaches.
- Vascular headache.

Vascular headaches are the results of dilatation of the intracranial and extra cranial arteries and are sometimes classed as migraine and non- migraine types.

Migraine is a mysterious disorder which is associated with nausea, vomiting, pain, sensitivity to light and sound, vertigo, loose motions and other symptoms.

As there is no established method for the simultaneous estimation of sumatriptan succinate, metoclopramide hydrochloride and paracetamol drugs in combined dosage form by RP HPLC, the present work describes the development of a validated RP-HPLC method, which can quantify these components simultaneously from a combined dosage form.

EXPERIMENTAL

Reagents and chemicals:

Acetonitrile and methanol (HPLC grade) was procured from Rankem Ltd., Delhi (India). Orthophosphoric acid (AR grade) were procured from Merck Ltd., Mumbai.

Potassium dihydrogen orthophosphate was procured from S.D Fine Chem Ltd., Mumbai. Water (HPLC grade) was obtained from a Milli-QRO water purification system. Reference standards of paracetamol was procured from Unichem pharmaceuticals, Mumbai, sumatriptan succinate was procured from Dr. Reddy laboratories Ltd, Hyderabad and metoclopramide HCl was procured from Ipca laboratories, Ratlam.

Apparatus and chromatographic conditions:

For the Chromatographic analysis, Shimadzu HPLC system was used that was equipped with a solvent delivery module {LC-10 AT VP}, Rheodyne manual injector 7725i fitted with 20 µL loop and UV detector {SPD-10A VP}. The separation was achieved on RP C18 column {125 x 4.6 mm, 5 micron particle size} using KH_2PO_4 buffer:MeOH (60:40 v/v) pH5 as mobile phase. The pH of mobile phase was adjusted to 5 with the help of N/20 KOH solution. The flow rate was 0.5 ml/min and the peaks were integrated by UV detector at230 nm for 6.8 min, 248 nm from 6.81 to 8.5 min and 213 nm for rest of the time. Operation, data acquisition and analysis were performed using Spinchrom 1.7 software. Analysis was performed at ambient temperature.

Preparation of standard solutions:

Standard stock solutions of 1000 µg/mL sumatriptan succinate, metoclopramide HCl and paracetamol, were prepared separately using acetonitrile. From the standard stock solution, mixed standard solution was prepared to contain100 µg/mL of each drug by using mobile phase solution.

Preparation of sample solution:

Three test solution of mixture of drugs were made. The three test solution represent three levels of concentration viz. High, medium, low. Concentration of 16 µg/ml, 4 µg/ml, 1 µg/ml represent the high, medium and low levels, respectively for sumatriptan succinate, paracetamol and metoclopramide HCl.

5 tablets of sumatriptan succinate and 10 tablets of each of paracetamol and metoclopramide HCl were weighed and

finely powdered. A quantity of the powder equivalent to one tablet was weighed and separately dissolved in 10 mL acetonitrile in a volumetric flask. All the flask were sonicated for 15 min twice and an aliquot was centrifuged at 1,000 rpm for 1 hour.

Assay method:

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention times of sumatriptan succinate, paracetamol and metoclopramide HCl were found to be 6, 7.5 and 10.8 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The response factor of the standard solution and sample solution were calculated.

RESULTS AND DISCUSSION

Estimation of sumatriptan succinate, metoclopramide and paracetamol in dosage forms:

The HPLC procedure was optimized with a view to develop precise and stable assay method. The pure drugs sumatriptan succinate, metoclopramide HCl and paracetamol were run in different mobile phase compositions with different pH and wavelength. The flow rate was also varied from 0.5 mL to 1.2 mL/min. Finally S.D fine chem. C18 column (125 x 4.6 mm, 5 micron particle size), with a mobile phase of a mixture of KH_2PO_4 buffer:MeOH (60:40 v/v) pH5. The pH of mobile phase was adjusted to 5 with the help of N/20 KOH solution. The flow rate was 0.5 ml/min and the peaks were integrated by UV detector at 230 nm for 6.8 min, 248 nm from 6.81 to 8.5 min and 213 nm for rest of the time. The typical chromatogram of sample solution is shown in Fig.1. The peak area ratio of standard and sample solutions was calculated. The percentage of individual drugs found in formulations, mean, standard deviation in formulations were calculated and presented in Table I. The results of analysis shows that the amounts of drugs were in good agreement with the label claim of the formulations.

Table – I: Results of Analysis of formulation and Recovery Studies

Drug	Quantity claimed (mg/tab)	Quantity found (mg/tab)	% Quantity found
Sumatriptan succinate	25	25.160	100.642
Paracetamol	500	499.97	99.99
Metoclopramide HCl	10	10.008	100.081

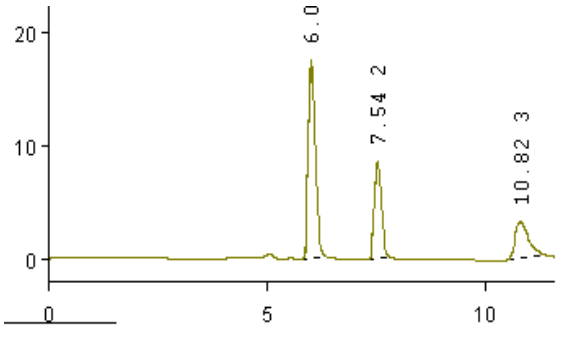

Fig 1. Typical Chromatogram of Sample Solution

Accuracy and precision:

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out three times and the percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained, added recoveries of standard drugs were found to be accurate.

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, three repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, three repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated. From the data obtained, the developed RP-HPLC method was found to be precise.

Linearity and Range:

The linearity of the method was determined at seven concentration levels ranging from 0.5 to 32 µg/mL for each of the three drugs. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 234.65x-23.465 (R^2=1) for sumatriptan succinate and y=167.37x-41.02 (R^2=0.999) for metoclopramide HCl and y=166.07x-20.02 (R^2=0.999) for paracetamol. The results shows that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above. The calibration curves are shown in following Figures. 2a,2b,2c..

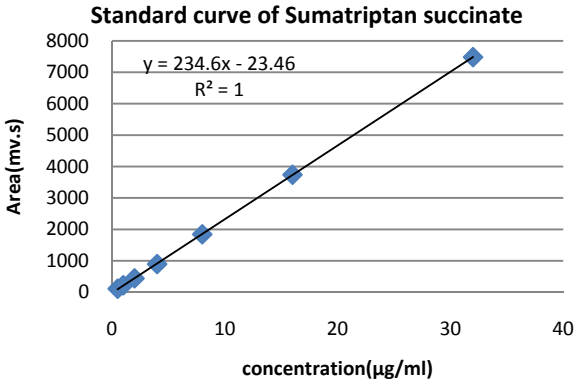

Fig 2a

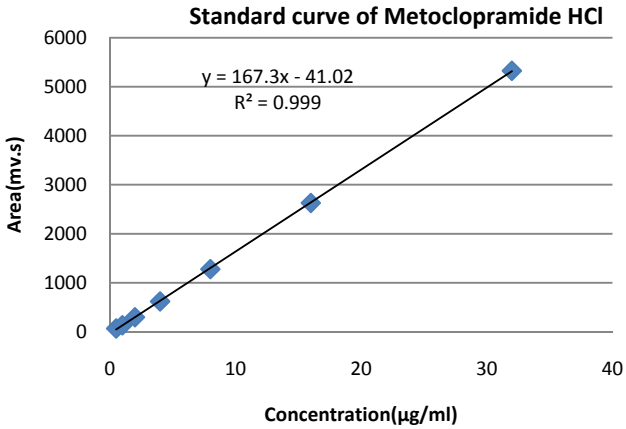

Fig 2b

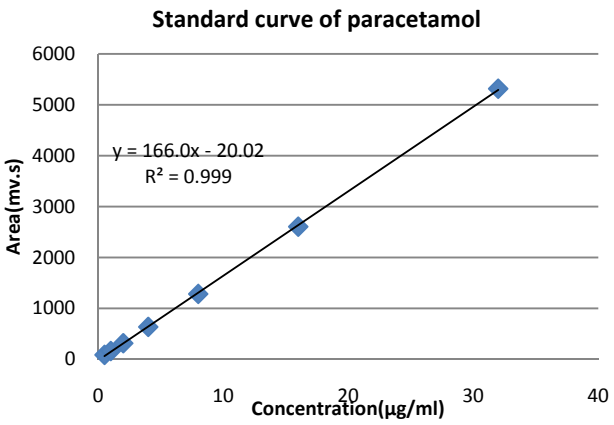

Fig 2c

Limit of Detection and Limit of Quantification:

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for sumatriptan succinate, metoclopramide HCl and paracetamol was found to be 0.0107µg/mL 0.0102 µg/mL and 0.0104 µg/mL respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 0.035 µg/mL for sumatriptan succinate and 0.034 µg/mL for metoclopramide HCl and paracetamol (Table II).

Table II: System Suitability Studies

Parameters	Sumatriptan succinate	Metoclopramide HCl	Paracetamol
Linearity range	0.5-32µg/mL	0.5-32µg/mL	0.5-32µg/mL
Regression equation Y = mx + c*	y = 234.65x-23.465	y=167.37x-41.02	y=166.07x-20.02
Correlation coefficient	1	0.999	0.999
Theoretical plate no.	6541	10271	5478
Resolution factor	-	5.117	7.401
Asymmetric factor	1.526	1.450	2.219
LOD (µg/mL)	0.0107	0.0102	0.0104
LOQ (µg/mL)	0.035	0.34	0.34

System suitability studies:

The retention time, capacity factor**,** theoretical plate no., resolution and peak asymmetry were calculated for the standard solutions (Table II). The values obtained demonstrated the suitability of the system for the analysis of these drug combinations.

CONCLUSION

The proposed RP-HPLC method for the simultaneous estimation of sumatriptan succinate, metoclopramide HCl and paracetamol in combined dosage forms is accurate, precise, linear, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolution studies.

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