An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

DEVELOPMENT AND VALIDATION OF Q-ABSORBANCE RATIO SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF SUMATRIPTAN AND PROMETHAZINE IN SYNTHETIC MIXTURE.

Nazneen Patel*, Dr. Jignesh S. Shah, Dr. Dilip Maheshwari

L.J. Institute of Pharmacy, Nr. Sanand Cross Roads, Sarkhej-Gandhinagar Highway, Ahmedabad Gujarat-382210, INDIA

***Corresponding author E-mail:** nazneenpatel2@gmail.com

ARTICLE INFO

Key Words
Sumatriptan, Promethazine, Q-Absorbance Ratio, Simultaneous Estimation

ABSTRACT

An accurate, simple, precise and reproducible UV-spectrophotometric method was developed and validated for simultaneous determination of Sumatriptan and Promethazine in synthetic mixture. Spectrophotometric estimation of Sumatriptan and Promethazine was carried out at 237nm and 226nm by Q Absorbance ratio method using distilled water as solvent. Linearity was performed over the concentration range of 4-24µg/ml and 2-12µg/ml for Sumatriptan and Promethazine respectively with correlation coefficient 0.999 for both the drugs. The developed method was validated according to ICH(Q_2R_1) guidelines and whereby %RSD values were found to be less than 2% complying the validation requirements. Method can be applied for routine analysis of Sumatriptan and Promethazine in Synthetic mixture.

INTRODUCTION:

Sumatriptan is a drug belonging to the class of triptan. It is use for the migraine with and without aura and for cluster headaches (fig.1). It is a 5-Hydroxy tryptamine (5-HT) receptor agonist type (5-HT_{1B} and 5-HT_{1D}) similar like the structure of serotonin. At the 5-HT1B/1D receptors on sensory nerves and intracranial blood vessels of the trigeminal system, it exerts agonist effect which result in inhibition of pro-inflammatory neuropeptide release and cranial vessel constriction [2]. It works by 3 mechanisms of action: vasoconstriction of the dilated vessels, inhibiting the nociceptive transmission in the trigeminal nerves system preventing the central sensitization development [3].

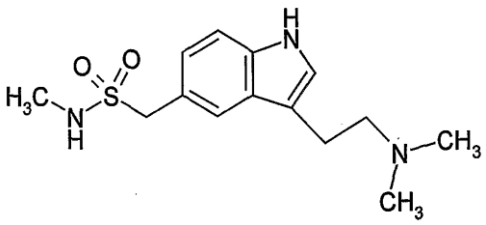

Fig.1: chemical structure of sumatriptan

Promethazine is an antiemetic drug belonging to the class of phenothiazine(fig.2). It is use in nausea, vomiting, in treatment of migraines, as a sedative for better sleep, pain reliever. It works as a strong H_1 receptor antagonist and a moderate mAch receptor antagonist(anticholinergic). It competes with free histamine for binding at the H_1

receptor sites present in large blood vessels, GIT, uterus. The relief of nausea appears to be related to central anticholinergic actions. It also blocks D_2 receptors in Limbic system and Basal ganglia specifically the D2-artery vasodilation [3].

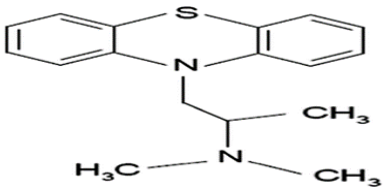

Fig.2: Structure of promethazine

Combination of sumatriptan plus promethazine was studied in clinical trial for the migraine associated with the nausea and vomiting compare to the sumatriptan monotherapy [3]. Sumatriptan plus Promethazine was proved to be effective at a dose of 2:1 in management of migraine compare to the sumatriptan monotherapy. Addition of promethazine to sumatriptan was proved effective in relief of nausea and vomiting, rate of pain recurrence reduced with significant improvement in the headache free rate at 4 hours compare to sumatriptan monotherapy. A triptan plus antiemetic showed the effective results in relief of the migraine associated Headache, Nausea, Vomiting, Photophobia and Phonophobia [3]. The present study focuses on the method development and validation of sumatriptan and promethazine in synthetic mixture. A successful attempt has been made to estimate two drugs simultaneously by Q Absorbance ratio spectrophotometric method.

MATERIALS AND METHODS:

Chemical and reagents

Sumatriptan and Promethazine were procured as generous gift sample by Intas Pharmaceuticals, Ahmedabad, Gujarat and Swiss Pharmaceuticals, Ahmedabad, Gujarat. Distilled water was used as solvent.

Instrumentation

A shimadzu model 1800 (Japan) UV-Visible double beam spectrophotometer is used with spectral width 2 nm, wavelength accuracy of 0.5 nm and pair of 10 mm matched quartz cell. Spectra was automatically obtained by UV probe system software (UV probe version 2.31).

Analytical method development

Selection of solvent

A suitable solvent is selected on the basis of stability and solubility of the drug in solvent system. The distilled water is found to be suitable solvent as both the drugs are freely soluble in distilled water [4].

Preparation of standard stock solution (100µg/ml)

Accurately weighed 10 mg of sumatriptan and promethazine and transferred into separate 100ml volumetric flask, dissolved to half, sonicated and made up to the mark with Distilled water. (100µg/ml)

Determination of wavelength:

0.8ml working standard stock solution of sumatriptan (100 µg/ml) and 0.4ml working standard stock solution of promethazine (100 µg/ml) was transferred in to different 10 ml volumetric flask and dilute up to mark with Distilled water to get 8µg/ml and 4µg/ml of sumatriptan and promethazine. Each solution was scanned in the range of 200-400 nm. From the overlain spectra of sumatriptan and promethazine, it is evident that sumatriptan and promethazine shows an isoabsorptive point at 237nm. The second wavelength used is 226nm, which is the λmax of sumatriptan.

Calibration Curve for sumatriptan (4-24 µg/ml):

An aliquots of stock solution of sumatriptan (100 µg/ml) 0.4, 0.8, 1.2, 1.6, 2, 2.4 ml was pipette out in 6 different 10ml volumetric flask and was made up to the mark with Distilled water to get 4, 8, 12, 16, 20, 24µg/ml respectively.

Absorbance of each solution was measured at 237nm (isoabsorptive point) and 226nm (λmax of sumatriptan) using Distilled water as blank. Graph of Absorbance vs. Concentration (µg/ml) was plotted.

Calibration Curve for promethazine: (2-12µg/ml)

An aliquots of stock solution of promethazine (100 µg/ml) 0.2, 0.4, 0.6, 0.8, 1, 1.2 ml was pipette out in 6 different 10ml volumetric flask and was made up to the mark with Distilled water which will give 2, 4, 6, 8, 10, 12 µg/ml respectively. Absorbance of each solution was measured at 237nm (isoabsorptive point) and 226nm (λmax of sumatriptan) using Distilled water as blank Graph of Absorbance vs. Concentration (µg/ml) was plotted.

Method validation of UV spectroscopic method: The developed method was validated with respect to linearity, accuracy, precision, limit of detection and limit of quantification in accordance with the ICH guideline(Q_2R_1) [7].

Linearity and range (n=6): The linearity of sumatriptan and promethazine was found to be in the range of 4-24 µg/ml and 2-12 µg/ml, respectively. Linearity of both the drugs was checked in term of slope, intercept and correlation coefficient.

Precision

Intraday precision (n=3): Solution containing sumatriptan 4, 8, 12µg/ml and promethazine 2, 4, 6µg/ml were analyzed three times on the same day and %R.S. D was calculated.

Interday precision (n=3): Solution containing sumatriptan 4, 8, 12µg/ml and promethazine 2, 4, 6µg/ml analyzed three times on analyzed on three different successive days and %R.S. D was calculated.

Repeatability (n=6):

Solutions containing of 8µg/ml of sumatriptan and 4µg/ml promethazine were analyzed three times on the same day and % R.S.D. was calculated. %R.S. D was not more than 2%.

Limit of detection (LOD)

The L.O.D. was estimated from the set of calibration curves of sumatriptan and promethazine used to determine method linearity. Limit of detection can be calculated using following equation as per ICH guidelines.

LOD = 3.3 σ/S

Where,

σ = Standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

Limit of quantitation: The L.O.Q. was estimated from the set of calibration curves of sumatriptan and promethazine used to determine method linearity. Limit of quantitation can be calculated using following equation as per ICH guidelines.

LOQ = 10 σ/S

Where,

σ = Standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

Accuracy (recovery study) (n=3)

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as conventional the value or an accepted reference value found. Accuracy of the developed method concentration levels 50%, 100%, 150%.

Preparation of sample solution for accuracy: Accurately weighed powder equivalent to 8mg of sumatriptan and 4mg Promethazine from the prepared synthetic mixture and transferred in 100 ml volumetric flask and make up to the mark with Distilled water. This solution was sonicated and filtered. The mixture contains sumatriptan 80µg/ml and promethazine 40µg/ml. From the above synthetic mixture 1 ml was pipetted out in 3 different 10ml volumetric flask and spiked with standard stock solution of sumatriptan and promethazine in 50, 100, 150 level. made up to the mark with Distilled water to obtain final concentration.

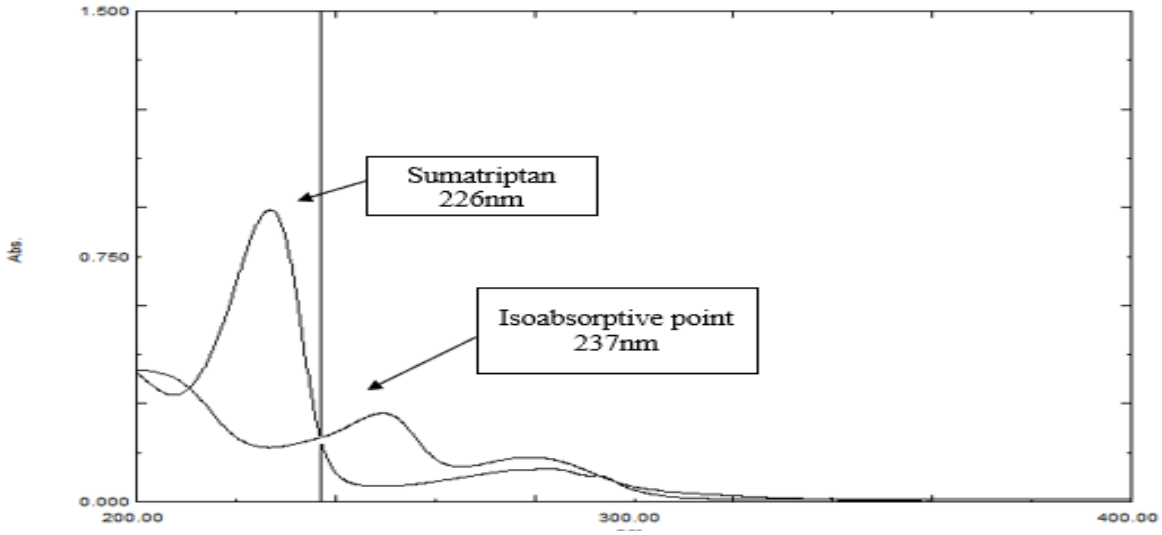

Fig.3: overlain spectra of sumatriptan (8µg/ml) and promethazine (4µg/ml) in distilled water.

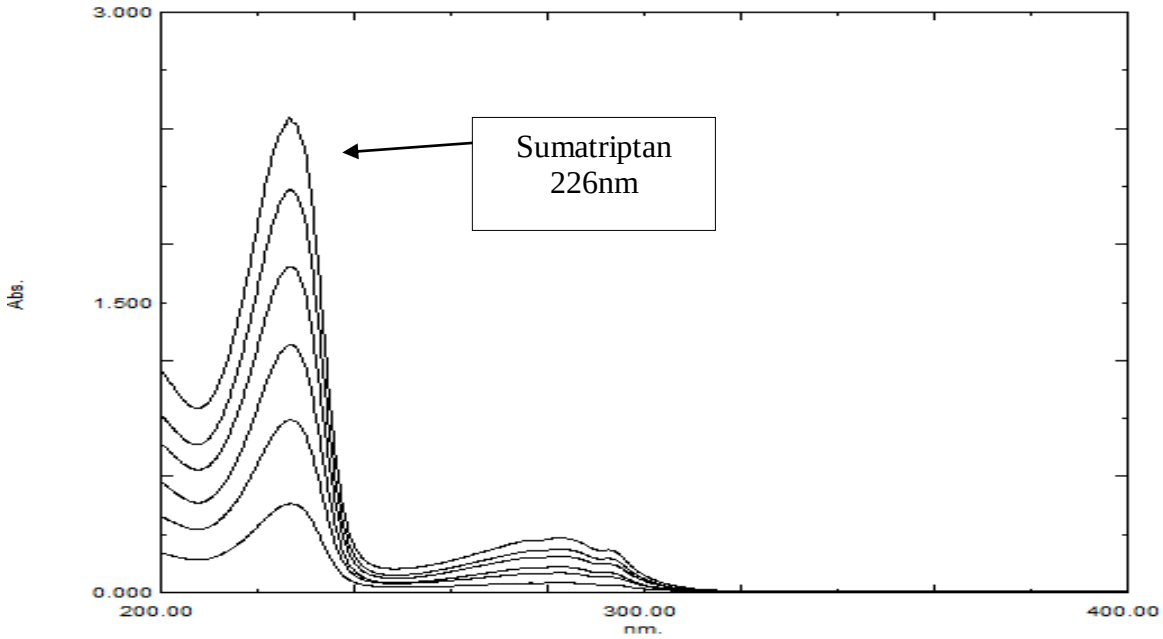

Fig.4: overlain spectra of Sumatriptan in distilled water (4-24µg/ml)

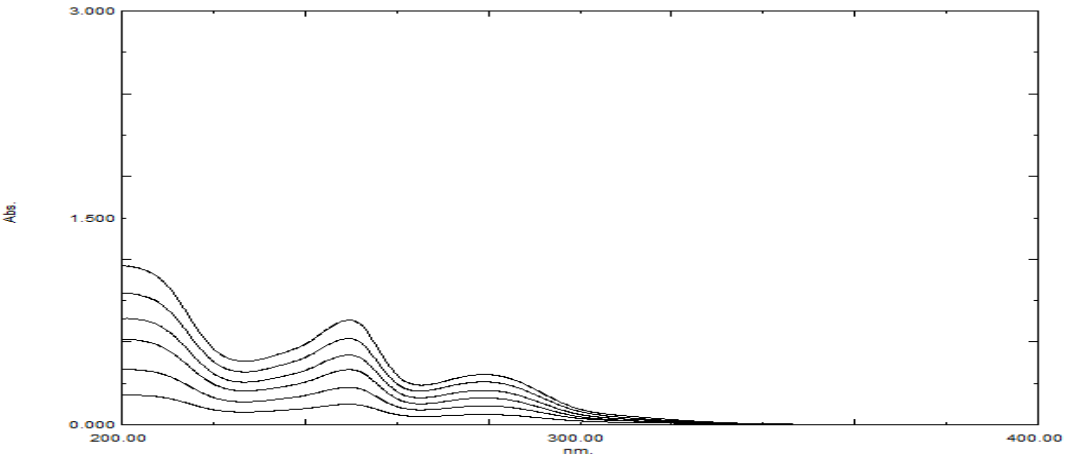

Fig.5: overlain spectra of Promethazine in distilled water (2-12µg/ml)

LINEARITY AND RANGE:

Table 1: Linearity data of sumatriptan at 226nm and 237nm

Sumatriptan (226 nm)				Sumatriptan (237 nm)		
S.no	Conc. (µg/ml)	absorbance± SD (n=6)	% RSD	Conc. (µg/ml)	absorbance±SD (n=6)	% RSD
1	4	0.459±0.00473	1.03	4	0.108±0.00178	1.64
2	8	0.861±0.00697	0.81	8	0.195±0.00299	1.53
3	12	1.289±0.00786	0.60	12	0.286±0.00326	1.14
4	16	1.685±0.00861	0.51	16	0.366±0.00389	1.06
5	20	2.081±0.00898	0.43	20	0.454±0.00413	0.90
6	24	2.451±0.00843	0.34	24	0.543±0.00422	0.77

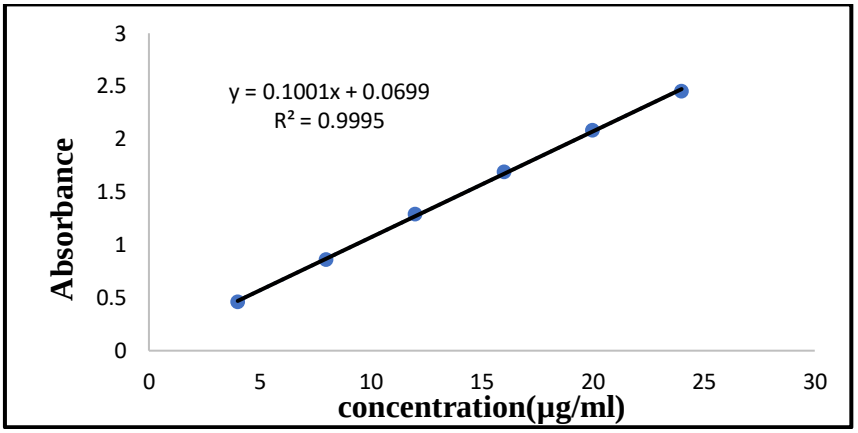

Fig.6: calibration curve of sumatriptan at 226 nm

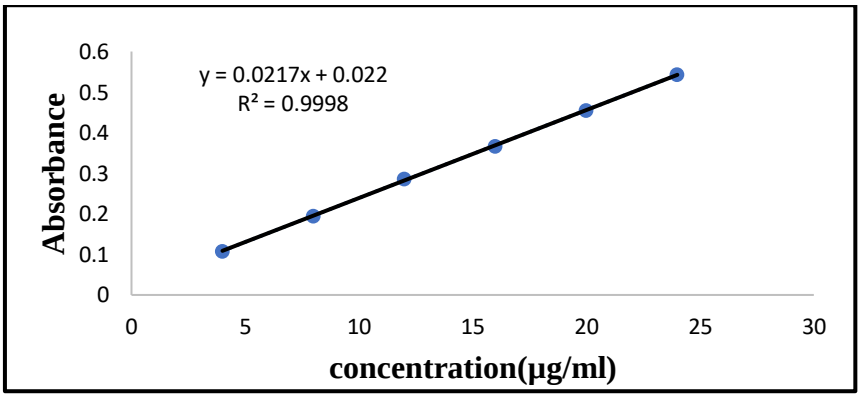

Fig.7: calibration curve of sumatriptan at 237 nm

Table 2: Linearity data of promethazine at 226nm and 237nm

Promethazine (226 nm)				Promethazine (237 nm)		
S.no	Conc. (µg/ml)	absorbance± SD (n=6)	% RSD	Conc. (µg/ml)	absorbance± SD (n=6)	% RSD
1	2	0.0931±0.00172	1.84	2	0.108±0.00178	1.64
2	4	0.166±0.00256	1.53	4	0.195±0.00299	1.53
3	6	0.246±0.00331	1.34	6	0.286±0.00326	1.14
4	8	0.306±0.00299	0.97	8	0.366±0.00389	1.06
5	10	0.384±0.00326	0.84	10	0.454±0.00413	0.90
6	12	0.464±0.00334	0.72	12	0.543±0.00422	0.77

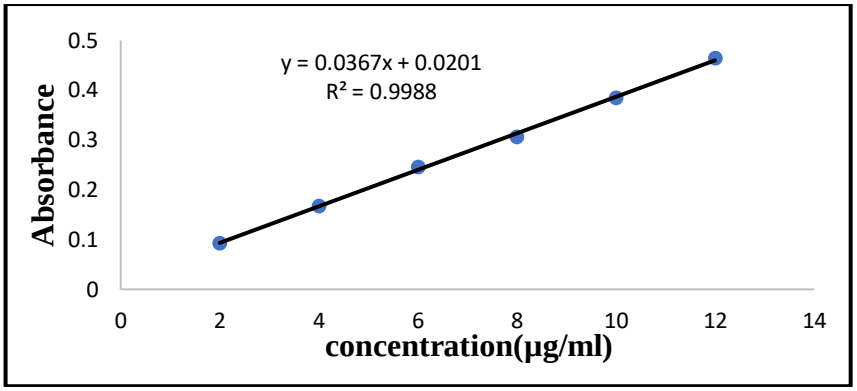

Fig. 8 calibration curve of promethazine at 226 nm

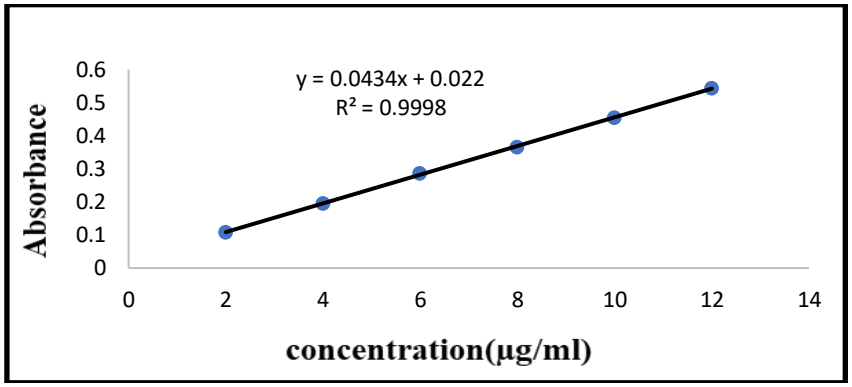

Fig. 9 Calibration curve of promethazine at 237 nm

PRECISION:

Table 3: Precision study of sumatriptan at 226 nm

Interday Precision of sumatriptan (n=3)			
S.no	Conc. (µg/ml)	Mean Absorbance ±SD	% RSD
1	4	0.457±0.0065	1.434
2	8	0.864±0.0087	1.010
3	12	1.286±0.0095	0.741
Intraday Precision of Sumatriptan (n=3)			
S.no	Conc. (µg/ml)	Mean Absorbance ±SD	% RSD
1	4	0.454±0.0043	0.960
2	8	0.865±0.0066	0.769
3	12	1.284±0.0070	0.546
Repeatability of Sumatriptan (n=6)			
S.no	Conc. (µg/ml)	Mean Absorbance ±SD	% RSD
1	8	0.866±0.0064	0.744

Table 4: Precision study of promethazine at 226 nm

Interday Precision of promethazine (n=3)			
S.no	Conc. (µg/ml)	Mean Absorbance ±SD	% RSD
1	2	0.097±0.00152	1.569
2	4	0.165±0.00208	1.256
3	6	0.247±0.00251	1.016
Intraday Precision of promethazine (n=3)			
S.no	Conc. (µg/ml)	Mean Absorbance ±SD	% RSD
1	2	0.095±0.0010	1.052

2	4	0.168±0.00152	0.905
3	6	0.245±0.00173	0.706
Repeatability of promethazine (n=6)			
S.no	Conc. (µg/ml)	Mean Absorbance ±SD	% RSD
1	4	0.166±0.00147	0.885

Table 5: Precision study at isoabsorptive point 237 nm

Interday Precision (n=3)				
S.no	Conc. (µg/ml) Sumatriptan	Conc. (µg/ml) Promethazine	Mean Absorbance ±SD	% RSD
1	4	2	0.107±0.00208	1.93
2	8	4	0.196±0.00321	1.63
3	12	6	0.285±0.0040	1.40
Intraday Precision (n=3)				
S.no	Conc. (µg/ml) Sumatriptan	Conc. (µg/ml) Promethazine	Mean Absorbance ±SD	% RSD
1	4	2	0.108±0.00152	1.41
2	8	4	0.198±0.00208	1.04
3	12	6	0.286±0.00264	0.92
Repeatability (n=6)				
S.no	Conc. (µg/ml) Sumatriptan	Conc. (µg/ml) Promethazine	Mean Absorbance ±SD (n)	% RSD
1	8	4	0.197±0.00195	0.98

LOD and LOQ:

Table 6: LOD and LOQ for sumatriptan and promethazine

Parameter	Sumatriptan	Promethazine
LOD (µg/ml)	0.123	0.223
LOQ (µg/ml)	0.375	0.678

ACCURACY:

Table 7: Recovery study

% Level of recovery	%Recovery± SD sumatriptan(n=3)	%Recovery± SD promethazine
50	98.08±0.207	99.00±0.140
100	99.43±0.249	99.37±0.316
150	99.80±0.332	99.80±0.422

ASSAY:

Table 8: Analysis of synthetic mixture

Name of drug	Amount taken (µg/ml)	Mean Amount found (µg/ml)	%Assay± SD (n=3)	%RSD
Sumatriptan	8	7.91	98.87±0.180	0.182
Promethazine	4	3.94	98.50±0.264	0.268

SUMMARY OF VALIDATION PARAMETER:

Table 9: Summary of validation parameter

Parameters	Sumatriptan		Promethazine	
λ_{max} (nm)	226	237	226	237

Linearity range(µg/ml)	4-24	4-24	2-12	2-12
Standard Regression equation	y = 0.1001x	y = 0.0217x	y = 0.0367x	y = 0.0434x
Intercept	0.0699	0.022	0.0201	0.022
Correlation Coefficient (r^2)	0.9995	0.9998	0.9988	0.9998
%Intraday Precision	0.75	1.12	0.88	1.12
%Interday Precision	1.06	1.65	1.28	1.65
%Recovery	99.10		99.36	
LOD (µg/ml)	0.123		0.223	
LOQ (µg/ml)	0.375		0.678	
Assay%	98.87		98.50	

Application of method to synthetic mixture:

Preparation of synthetic mixture: The synthetic mixture of Sumatriptan and Promethazine was prepared in ratio of 2:1. The API taken as Sumatriptan 50mg and Promethazine 25mg. Common excipients: Microcrystalline cellulose, Lactose, Magnesium stearate, Talc [8]. Accurately weighed powder equivalent to 8mg of Sumatriptan and 4mg Promethazine from the prepared synthetic mixture and transferred in 100 ml volumetric flask and make up to the mark with Distilled water. This solution was sonicated and filtered. The mixture contains Sumatriptan 80µg/ml and Promethazine 40µg/ml.

Preparation of sample solution: From the above synthetic mixture 1 ml was pipetted out in 10ml volumetric flask and made up to the mark with Distilled water to obtain final concentration of Sumatriptan 8µg/ml and Promethazine 4µg/ml. The concentration of each drug was calculated using equation of Q-absorption ratio method.

DISCUSSION:

This study represents the development and validation of simple U.V method for the determination of Sumatriptan and Promethazine in synthetic mixture. The method is less time consuming and the sensitivity of the method is high. The %assay of Sumatriptan and Promethazine by absorbance ratio method was found to be 98.87 and 98.50 respectively. The U.V method developed and validated for the determination of Sumatriptan and Promethazine in synthetic mixture, assured the satisfactory precision and accuracy. The method was found to be simple, accurate and precise as per ICH guidelines. The method was successfully used for determination of drugs in synthetic mixture.

CONCLUSION:

A simple, accurate and precise Q-Absorbance method has been developed and validated for routine analysis of Sumatriptan and Promethazine. The developed method is recommended for routine and quality control analysis of both the drugs in synthetic Mixture

ACKNOWLEDGMENT

The authors are highly thankful to Dr. K. Pundarikakshudu, Director of L. J. Institute of Pharmacy, Ahmedabad, India for providing valuable guidance and all the facilities to carry out the research work.

REFERENCES:

1. Prameela B, Subhashini S, Anusha V, Eswar TD and Rama RN. Migraine – A Malady A Short Review. *Sch. Acad. J. Pharm.* 2014; 03(3):285-289.
2. Tepper SJ, Rapoport AM and Sheftell FD. Mechanism of action of the 5-HT1D/1B receptor agonist. *Arch Neurol* 2002; 7:1084-1088
3. Shadi A, Kamran H, Reza V, Mohammad MF, Afshin A and Ali S. Promethazine Plus Sumatriptan in the Treatment of Migraine: A Randomized Clinical Trial. Headache American Society, Wiley Periodicals 2014; 94-107.

4. Maryadele NJ. The Merck Index an Encyclopedia of chemicals drugs and biological. Merck Research Laboratories. 2006. p. 1339,1544
5. S. Ravi, Y. Darwis, N. Khan. Development and Validation of an RP-HPLC– UV Method for Analysis of Sumatriptan Succinate in Pharmaceutical Dosage Forms. *Acta Chromatographica* 2009; 3:421–432.
6. Gondalia R and Abhay D. Simultaneous Estimation of Sumatriptan Succinate and Naproxen Sodium in Bulk Drug and Pharmaceutical Dosage Form By RP-HPLC. *Journal of Drug Delivery and Therapeutics* 2013; 3(2):93-97.
7. ICH Topic Q2A, Validation of Analytical Methods: Definitions and Terminology, Step 5, CPMP/ICH/381/95.
8. Rowe RC, Sheskey PJ and Quinn ME. Handbook of Pharmaceutical Excipients. Pharmaceutical press, London, 2009. p. 210-214, 404-407, 663-666, 728-730.
9. Beckett AH and Stenlake JB. Practical Pharmaceutical Chemistry. CBS Publication, 2001. pp. 87.