



Development and validation of RP-HPLC-PDA method for the analysis of sumatriptan in *in vitro* transdermal permeation studies

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ABSTRACT

The aim of the present work is to develop and validate a simple, efficient, economical RP-HPLC method for the analysis of Sumatriptan (SMT) in samples obtained from *in vitro* transdermal permeation studies. A Phenomenex C₁₈ reverse phase column (150 x 4.6mm, 5µm) with mobile phase containing 10mM ammonium acetate: methanol (75:25% v/v) at a flow rate of 1.2mL/min was used in isocratic mode and eluents were monitored at 230nm. The retention time of SMT was 3.61 min and showed a good linearity in the concentration range of 0.2-3µg/mL with a correlation coefficient >0.999. *In vitro* skin permeation studies were carried out using a Franz diffusion Cells. Absence of interference peaks at the retention time of SMT indicates that the method was specific for the analysis of SMT in samples obtained from *in vitro* transdermal permeation studies. The percent recoveries were ranged in between 98-102 (RSD < 2). The validation parameters like specificity, linearity, accuracy and limit of detection, limit of quantification, precision, robustness fulfilled regulatory requirements. The developed HPLC method was successfully used for the analysis of SMT in samples obtained from *in vitro* transdermal permeation studies.

Keywords: C₁₈ reverse phase column, Method validation, PDA detection, Sumatriptan, Transdermal permeation studies.

INTRODUCTION

SMT is a cholinesterase inhibitor and chemically it is 1-[3-(2-dimethyl amino ethyl)-1H-indol-5yl]-N-methyl-methane sulfonamide and used in the treatment of migraine headaches [1]. It is a 5-HT_{1B/1D/1F} receptor agonist, it acts by inhibiting trigeminal nerve transmission and it also constricts large arteries [2].

Various analytical methods have been reported in the literature for quantitative determination of SMT in combination with other drugs like Naproxen, Domperidone, by HPLC [3-6], spectrophotometry [7-9], fluorimetry [10-11], and by LC-MS [12-13]. Literature survey reveals that there were no validated RP-HPLC-PDA methods reported for the estimation of SMT in transdermal permeation studies. Hence, the present investigation was aimed at developing a validated RP-HPLC-PDA method for the analysis of SMT in samples obtained from *in vitro* transdermal permeation studies.

EXPERIMENTAL SECTION

Chemicals

SMT was gift sample from Aurobindo Pharma Ltd, Hyderabad, India. Ammonium acetate, water and methanol were purchased from E. Merck, Mumbai, India. Sodium chloride was purchased from SDFCL, Mumbai, India. All the solvents and reagents were of HPLC grade.

Equipment

A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20AHT auto sampler, and SPD-M20A PDA detector was used. Data acquisition was carried out using LC solutions software. The chromatographic analysis was performed on C₁₈ reverse phase Phenomenex column (150 × 4.6mm, 5μ).

In Vitro Skin Permeation Studies

The *in vitro* transdermal permeation studies were performed using a vertical type Transdermal Diffusion Cell apparatus with a water circulation system, a water heater and an eight stage magnetic stirrer (Orchid Scientifics, Nashik, India). Franz diffusion cells with a diffusion area of 1.77 cm² and a receptor volume of ~14 mL were used and 0.9% w/v sodium chloride (saline) was used as receptor fluid. In the case of skin permeation studies the porcine ear skin was mounted between donor and receptor chambers facing stratum corneum surface towards donor side and clamped. The surface of the skin was maintained at 32°C by keeping receptor fluid at 37°C using a circulation water bath. 500 μL of saturated SMT solution in propylene glycol: saline (70:30 v/v) was added to donor chamber. Samples of 500 μL were withdrawn at pre-determined time intervals and stored at 4°C until analysis. 500 μL of fresh saline was replaced at each sampling point. The study was carried out over a 48 hr period and the samples were analyzed for SMT content by developed HPLC method.

Chromatographic Conditions

Mobile phase consisting of 10mM ammonium acetate: methanol (75:25% v/v) was used in isocratic mode and the mobile phase was filtered through nylon disc filter of 0.45μm (Millipore) and sonicated for 3 min before use. The flow rate was 1.2 mL/min and the injection volume was 10μL. PDA detection was performed at 230nm and the separation was achieved at ambient temperature.

Preparation of stock and standard solutions

The stock solution of SMT strength 1mg/mL was prepared by dissolving 14 mg of Sumatriptan succinate (equivalent to 10mg of sumatriptan) in water and volume was adjusted to the mark with the same. An appropriate volume of the stock solution was then further diluted with 0.9% w/v Sodium chloride to get the required concentrations of standard solutions of SMT at a concentration range of 0.2-3μg/mL.

Validation of the HPLC method

The proposed method was validated as per ICH guidelines.

Linearity

A linear relationship was evaluated across the range of the analytical procedure with a minimum of five concentrations. A series of standard dilutions of SMT were prepared over a concentration range of 0.2-3μg/mL (0.2, 0.5, 1, 2, 3μg/mL) from stock solution and injected in triplicate. Linearity is evaluated by a plot of peak areas as a function of analyte concentration, and the test results were evaluated by appropriate statistical methods where by slope, intercept, and regression (R²) correlation coefficients (R) were calculated and the data was given in Table 1.

Precision

Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions. Repeatability was assessed by using a minimum of six determinations at 100% of the test concentration. The standard deviation and the relative standard deviation were reported for precision. Less than 2% RSD for peak areas indicates the precision of the developed method and the data was presented in Table-1.

Specificity

The specificity of the method was determined by comparing the chromatograms obtained from the drug substance with that obtained from the samples obtained from *in vitro* transdermal permeation samples using PDA detector. The overlay of diluent, standard and sample were presented in Figure-2. Absence of interference of excipients indicates the specificity of the proposed method.

Accuracy

Accuracy was established across the specified range of the analytical procedure. To ascertain the accuracy of the proposed method recovery studies were performed by the standard addition method by spiking 80%, 100%, 120% of the known quantities of standard within the range of linearity to the synthetic solution of diffusion sample (1μg/mL) and these solutions were analyzed by developed method in triplicate. The % recovery and the %RSD were calculated at each level of addition and the data was given in Table-1.

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

LOD and LOQ were calculated based on calibration curves. They were expressed as $LOD = (3.3 \times \sigma)/m$; $LOQ = (10.0 \times \sigma)/m$ (Where, σ is the standard deviation of the y-intercepts of the three regression lines and m is mean of the slopes of the three calibration curves).

Robustness

To determine the robustness of the method developed, the experimental conditions were deliberately altered and the chromatographic parameters viz., tailing factor, no. of theoretical plates were recorded. The flow rate of the mobile phase was 1.2mL/min. To study the effect of flow rate, the flow rate was changed by 20%, the effect of wavelength was studied by changing wavelength by ± 2 nm, the effect of mobile phase was studied by changing the mobile phase by 20% and the data was given in Table-2.

System suitability

System suitability was carried out by injecting a standard concentration at different injection volumes in the range of 10-50 μ L. The system suitability test parameters were noted and % RSD was calculated.

RESULTS AND DISCUSSION

Various HPLC-UV and LC-MS methods were published for the estimation of SMT in bulk and dosage form but so far no methods were reported on the quantification of SMT in transdermal permeation studies. Hence, the present investigation was aimed to develop a simple, economical RP-HPLC-PDA method for the determination of SMT in samples obtained from *in vitro* transdermal permeation studies.

Method Development

Initially mobile phase optimization was carried out with Inertsil ODS column (250 x 4.6mm, 5 μ) using water and acetonitrile (38:62% v/v) as mobile phase at a flow rate of 1 mL/min and 0.9% Sodium chloride was used as diluent and the eluents were monitored at 230nm. With these conditions, SMT was eluted with solvent front at 2.533 min. In another trial Phenomenex C₁₈ column (250 x 4.6mm, 5 μ) was used with formic acid (0.02% v/v) and methanol (25:75% v/v) as mobile phase at a flow rate of 1mL/min and with these conditions SMT peak was eluted after solvent front with retention time 3.464 min but the eluted peak was broad. Finally, the mobile phase of 10mM ammonium acetate and methanol (75:25% v/v) with Phenomenex C₁₈ column (150 x 4.6mm, 5 μ) was selected at a flow rate of 1.2mL/min using 0.9% sodium chloride as diluent and under these conditions sharp SMT peak was eluted at 3.61 min with good symmetry and tailing factor was within the limits. For quantitative purpose wavelength was set at 230 nm, which provided better reproducibility without interference. The peak purity index was found to be greater than 0.9999 and this indicating peak purity of the drug sample used in the analysis and the SMT chromatogram along with UV spectrum and peak purity profile was shown in Figure-1.

Method Validation

The method has been validated as per ICH-Guidelines for following parameters.

Linearity

The range of reliable quantification was set at the concentrations of 0.2-3 μ g/mL of SMT. This range was selected based on 80-120% of the standard concentration used for accuracy and were analyzed in triplicate. Peak areas and concentrations were subjected to least square regression analysis to calculate regression equation. The regression equation was $y = 64239x - 1362$ and correlation coefficient (R) was found to be 0.999 indicating a linear response over the range used. The data from the calibration curve was given in Table-1.

Precision

Precision studies were carried out in terms of repeatability. Repeatability of standard application was assessed by using six replicates of concentration at 1 μ g/mL level and the data was given in Table-1. The % RSD was found to be below 2 for peak areas, this shows the closeness of the data values to each other, indicating the precision of the method.

Accuracy

Accuracy of the proposed method was ascertained by performing recovery studies by standard addition method by spiking the known quantities of standard at 80%, 100%, 120% to the drug product solution of 1 μ g/mL and these solutions were analyzed in triplicate in each level of addition. The %RSD and the %Recovery were within the acceptable limit in all cases. It is evident from the results of accuracy study given in Table-1, that the proposed method enables very accurate quantitative estimation of SMT.

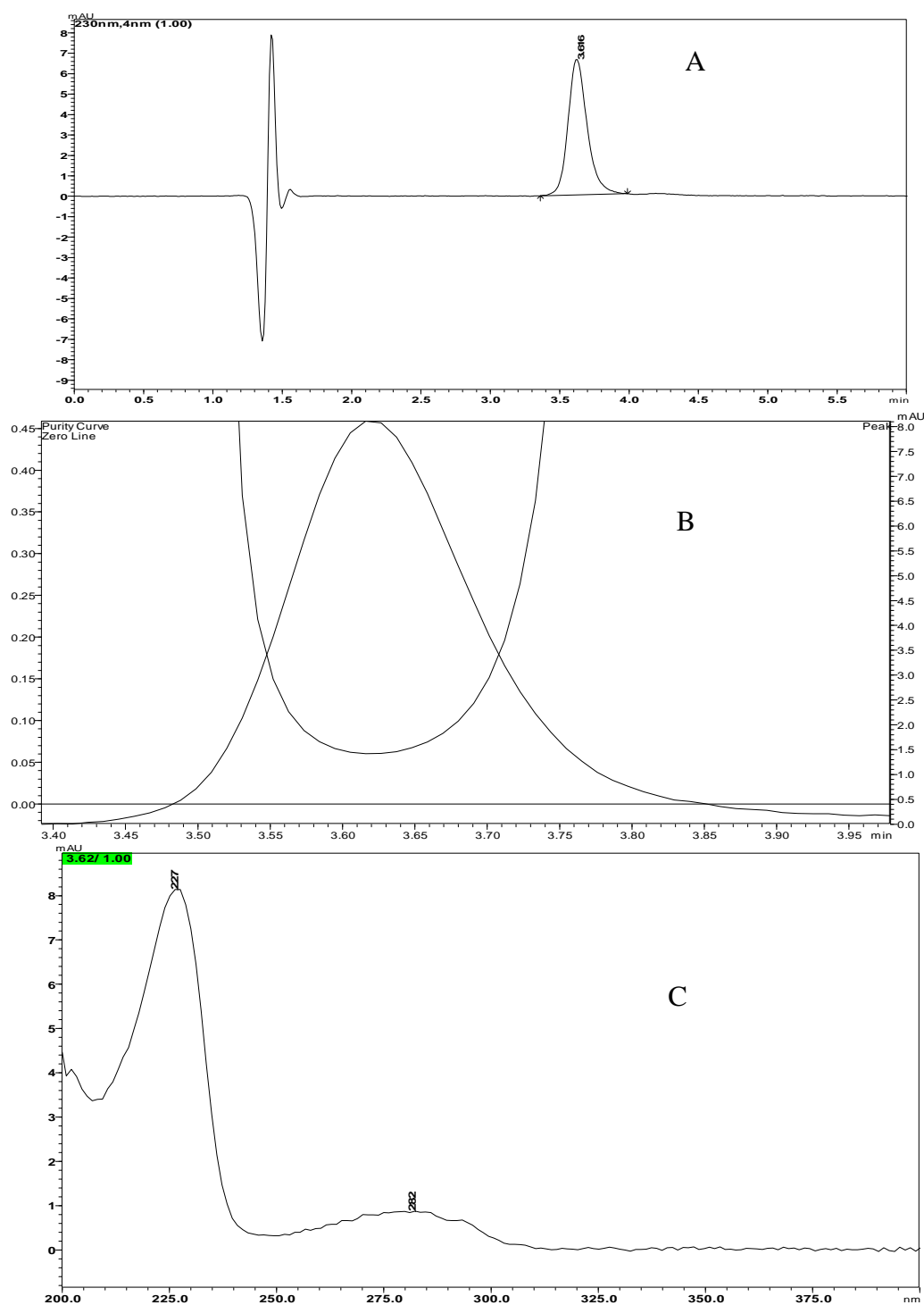


Fig. 1: A - Chromatogram of SMT (1µg/mL); B - Peak purity index of SMT and C - UV spectrum of SMT

Specificity

The specificity of the method was established by injecting the solutions of diluent, standard, diffusion sample individually to examine any interference, from the overlay of chromatograms as shown in (Figure-2) it can be inferred that there were no co-eluting peaks at the retention time of SMT. These results indicate that the peak of analyte was pure and the *in vitro* transdermal permeation samples did not show any interference with the sample analysis. The peak purity indices for sample and standard were found to be greater than 0.999 and this confirms specificity of the method by PDA detector.

Table-1: Linearity, Precision and Accuracy data of SMT

	Parameters	SMT
Linearity(n=3)	Range	0.2-3 μ g/mL
	Régression équation	Y=64239x-1362
	Régression coefficient (R ²)	0.999
	Corrélation coefficient (R)	0.999
Accuracy(n=3)	%Level of Addition	Mean Recovery(%RSD)
	80	99.86 (0.16)
	100	100.63(0.55)
	120	102.15(0.86)
Precision(n=6)		
System Precision	Average Peak area of the standard sample(%RSD)	62509 (0.94)
Method Precision	Average peak area of the Assay sample(%RSD)	63663.67(1.42)

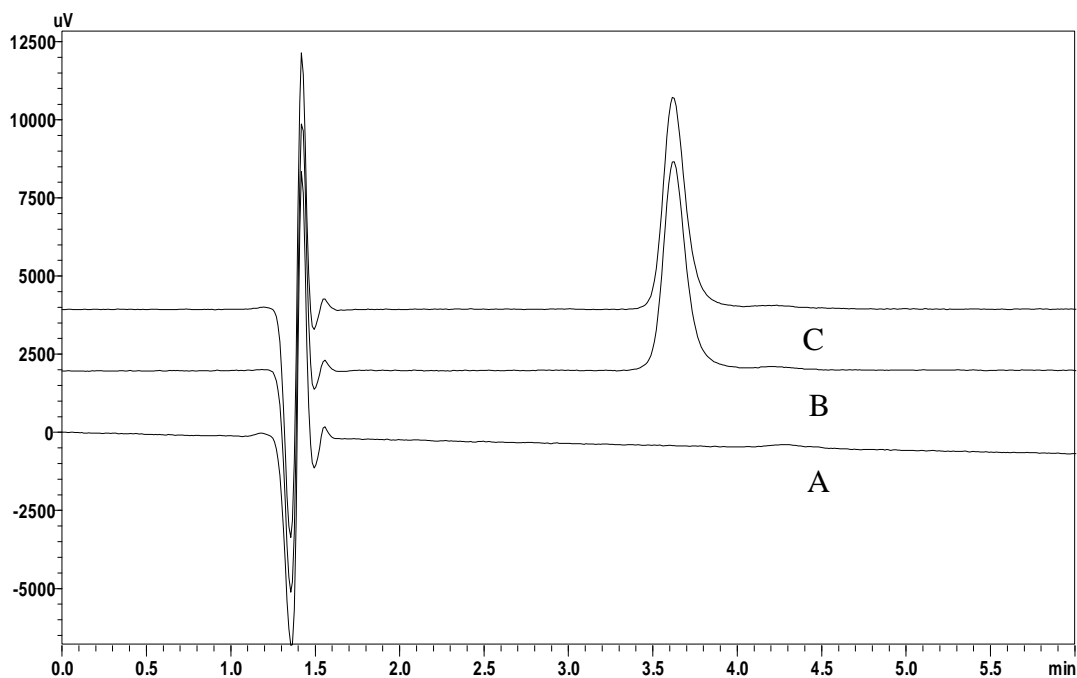


Fig. 2: Overlay of the Diluent (A), SMT Standard (B) and SMT Sample (C) chromatograms

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

LOD and LOQ were determined based on statistical calculation from the calibration curves, where $LOD = (3.3 \times \sigma)/m$; $LOQ = (10.0 \times \sigma)/m$ (σ is the standard deviation of the y-intercepts of the three regression lines and m is mean of the slopes of the three calibration curves). The limit of detection and limit of quantification were found to be 0.0134 μ g/mL and 0.0407 μ g/mL respectively and the SMT peaks were detected without any base line disturbances at this concentration.

Table-2: Robustness data of SMT:

Chromatographic parameter	Retention time (min)	Theoretical plates #	Tailing factor (T _f)
Flow rate (mL/min)			
1.0	3.949	3670.363	1.381
1.2	3.348	3326.996	1.366
1.4	2.900	3008.664	1.344
Wavelength(nm)			
228	3.348	3325.36	1.376
230	3.348	3327	1.377
232	3.348	3328.28	1.378
Mobile phase			
73:27	2.929	3090.565	1.355
75:25	3.348	3326.996	1.377
77:23	3.534	2454.786	1.388

Robustness

As part of the robustness, a deliberate change in the flow rate, wavelength and mobile phase was made to evaluate the impact on the method. Retention times were significantly changed with flow rate, mobile phase and no change in the retention time was observed in wavelength change. The parameters like theoretical plate number, tailing factor were not changed and were within the limits. These results indicated that the method is robust in terms of changed flow rate, mobile phase and wavelength.

System suitability

System suitability testing is an integral part of the analytical procedure. System suitability studies were carried out by injecting five times a 1 μ g/mL standard concentration of SMT at different injection volumes ranging from 10 μ L to 50 μ L. The %RSD values for system suitability test parameters like retention time [$R_t = 3.61$ (1.182%)], tailing factor [$T_f = 1.216$ (0.396%)] and theoretical plate number [$\# = 3452.4878$ (0.631%)] were less than 2% indicating the present conditions were suitable for the analysis of SMT in samples obtained from *in vitro* transdermal permeation studies.

Stability of the stock solution

The stability of the stock solution was determined by analyzing the samples under refrigeration ($8 \pm 1^\circ\text{C}$) at different time intervals up to 24hrs. The variation in assay values at different time intervals were found to be less than 2% of the initial zero time interval solution, thus indicating that the solutions were stable for a period of 48hrs when stored at 8°C .

***In vitro* transdermal permeation studies**

The validated method was used for the analysis of SMT in *in vitro* transdermal permeation studies. No interference peaks were observed with the SMT peak. Permeation profile was plotted with the cumulative amount permeated (nmol) versus time (hrs). The permeation profile was presented in Figure-3. From the permeation profile the transdermal flux in the steady state was found to be 283.40 nmol / cm² / hr and the lag time of 2.6 hrs. The amount of drug retained in the skin after the transdermal permeation study was also estimated by the developed method. The method can be successfully used for the quantification of SMT in the skin permeation studies.

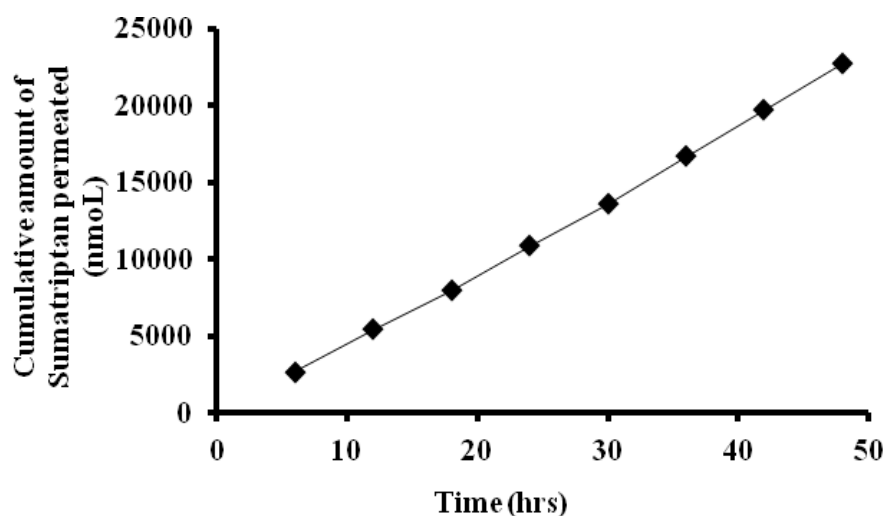


Fig.3: *In vitro* permeation profile of SMT

CONCLUSION

In this work, a simple and efficient RP-HPLC-PDA method was developed for the analysis of SMT in samples obtained from *in vitro* transdermal permeation studies. The method was validated fully as per International Conference on Harmonisation (ICH) Guidelines, and found to be applicable for routine quality control analysis for the estimation of SMT in samples obtained from *in vitro* transdermal permeation studies using isocratic binary mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The method provides selective quantification of SMT without interference from diluent. Therefore, this method can be employed in quality control to estimate the amount of SMT in samples obtained from *in vitro* transdermal permeation studies.

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