Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (5):205-210
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Development and validation of UV spectrophotometric method for simultaneous estimation of Tramadol hydrochloride and Quercetin in niosomes formulation

Shilpa P. Chaudhari*, Kavita Tawani and Paresh R. Mahaparale

Padmashree Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune, Savitribai Phule Pune University, Pune, India

---

ABSTRACT

Simultaneous estimation of Tramadol HCl and Quercetin (Dihydrate) using a specific, rapid and simple UV spectrophotometric method with good sensitivity in niosome formulation has been developed. From the optical characteristics of the proposed methods, it was found that the λ max of Tramadol HCl and Quercetin(Dihydrate) was found to 271 nm and 372 nm respectively. Tramadol HCl and Quercetin (Dihydrate) obey linearity within the concentration range of 0 – 20 µg/ml and 2-20 µg/ml. Recovery study was performed to confirm the accuracy of the method. According to ICH guidelines Method development and validation has been adopted for routine estimation of Tramadol HCL and Quercetin (Dihydrate) niosome formulation.

Key words: Tramadol HCL, Quercetin (Dihydrate), Simultenious equation, U.V. Spectrophotometer, validation.

INTRODUCTION

Tramadol hydrochloride is a centrally acting analgesic, used for treating moderate to severe pain. Tramadol hydrochloride possesses agonist actions at the µ-opioid receptor and effects reuptake at the noradrenergic and serotonergic systems. Tramadol is a compound with µ-Agonist activity, It is used to treat moderate to moderately severe pain and most types of Neuralgia, including trigeminal neuralgia.[1] Numerous epidemiological studies suggest a protective role of dietary flavonoids against cerebral ischemic disease. Flavonoids possess the cerebroprotective beneficial effect like antioxidant, anti-inflammatory, anti-platelet aggregatory activities and restore endothelial function and prevent LDL oxidation .Quercetin is found in redwine , green tea, onions, berries, citrus fruit, apples, garlic.[2] Quercetin has cerebroprotective effect and hence helps to reduce the side effects of tramadol hydrochloride if used in combination. There are various methods available for estimation of Tramadol hydrochloride like UV spectrophotometric, spectrofluorometry, HPLC , gas chromatography, GC-MS and LCMS, capillary electrophoresis, HPTLC, HPTLC-densitometry, etc.[3] for Quercetin U.V. HPTLC method reported .not a single U.V. method reported for simultaneous estimation of Tramadol HCL and Quercetin in formulation. Present research work is done to develop and validate a simple UV spectroscopy based method using simultaneous equation for rapid, accurate and precise estimation of Tramadol HCL and Quercetin in single formulation.

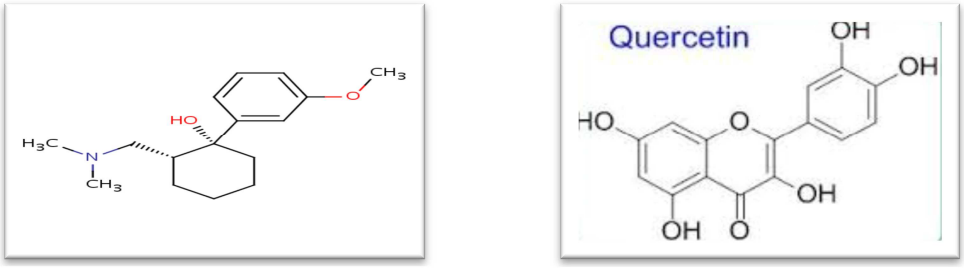

Figure 1.structure of (A) Tramadol HCL **(B) Quercetin**

MATERIALS AND METHODS

Apparatus

A double beam UV-spectrophotometer (Shimadzu, UV-1700, Japan), attached to a computer software UV Probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells, digital balance (Radwag.AS220/C/2 LC/GC), ultrasonicator (Steryl 40050, Mumbai, India), volumetric flasks and pipettes of borosilicate glass were used for the development and validation of proposed analytical method.

Material

Tramadol HCL gift sample from Glenmark pharmaceuticals (Mumbai) , Quercetin(Dihydrate) purchased from Otto Chemie Pvt.Ltd (Mumbai, Maharashtra). All the reagents used in this assay were of analytical grade and the reagent solutions were prepared using preanalysed distilled water, Distilled water was used as a solvent for the spectrophotometric estimation.

Procedures

Preparation of standard stock solution and calibration curve

Weighed an accurate amount 10mg of Tramadol hydrochloride was dissolved in 20ml methanol and diluted upto 100ml by distilled water to obtain a 100mcg/ml concentration of Tramadol hydrochloride in solution. Weighed an accurate amount 10mg of Quercetin(Dihydrate) was dissolved in 20ml methanol and diluted upto 100ml by distilled water to obtain 100mcg/ml concentration of Quercetin(Dihydrate) , This solutions was subjected to scanning between 200 – 400 nm and absorption maxima at 271nm and 372 nm for Tramadol hydrochloride and Quercetin(Dihydrate) respectively were determined.

Simultaneous equation method

From the overlain spectra (**Fig 2**) of tramadol HCL (10 µg/ml) and Quercetin(Dihydrate) (10 µg/ml), two wavelengths i.e. 271 nm as λ max of tramadol hcl and 372 nm as λ max of Quercetin(Dihydrate) were selected as the working wavelength, at which both drugs showed absorbance for each other. The absorptivity of these two drugs was determined at 271 nm and 372 nm. A set of two simultaneous equations were formed using absorptivity values as given in equation (1) and (2), at selected wavelengths. The concentrations of two drugs in niosome formulation were calculated using set of two simultaneous equations.

$$Cx = \frac{A2\ ay1 - A1ay2}{ax2\ ay1 - ax1\ ay2} \quad (1)$$

$$Cy = \frac{A1\ ax2 - A2\ ax1}{ax_2\ ay1 - ax1\ ay_2} \quad (2)$$

Where; Cx and Cy are concentrations of tramadol hydrochloride and Quercetin(Dihydrate) (µg/ml) respectively in known sample solution. A1 and A 2 are absorbance of sample solutions at 271 nm and 372 nm respectively. ax1 and ax2 are absorptivity of tramadol hcl at 271 nm and 372 nm, ay1 and ay2 are absorptivity of Quercetin(Dihydrate) at 271 nm and 372 nm. The concentration of Cx and Cy in niosome formulation can be

obtained by solving equation (1) and (2). Validity of above framed equation was checked by using mixed standard of pure drug sample of two drugs, measuring their absorbance at respective wavelength and calculating concentration of two components.

Analysis of the noisome formulation

Niosome solution (1 ml) containing equivalent to 10 mg of both drugs was transferred to 50 ml volumetric flask and excipient extracted with chloroform. Then drug dissolved in methanol. The sample solution was then filtered through Whatman filter paper. This solution was appropriately diluted to get approximate concentration of 20 µg/ml of Tramadol HCl and Quercetin , each, the absorbance of sample solution were measured at 271 nm and 372 nm against blank.

VALIDATION OF THE DEVELOPED METHOD

Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. The Beer-Lambert's concentration range was found to be 0-20 µg/ml for Tramadol hcl and 2-20 µg/ml for Quercetin(Dihydrate). The linearity data for method is presented in **Table1.**

Accuracy

Accuracy was confirmed by recovery study as per ICH norms at three different concentration levels 80%, 100%, 120% by replicate analysis (n = 3). Here to a preanalysed sample solution, standard drug solutions were added and then percentage of drug content was calculated. The result of recovery studies are reported in **table 3.**

Precision

Inter-day and Intra-day precision

An intermediate precision was carried out by intra and inter day precision study. In intra day study concentration drugs were calculated on the same day at an interval of one hour. In inter day study the drug contents were calculated on three different days. Study expresses within laboratory variation in different days.the result of same are presented in **Table 2**

Ruggedness Study

It expresses the precision within laboratories variations like different analyst. Ruggedness of the method was assessed by spiking the standard 3 times with different analyst by using same equipment. The results of the same are presented **in Table 5.**

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

The LOD and LOQ of Tramadol HCl and Quercetin(Dihydrate) by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3□/S and 10□/S respectively, where S is the slope of the calibration curve and is the standard deviation of response. The results of the same are shown in **Table 1**

RESULTS AND DISCUSSION

The UV scan of standard solution between 200 – 400 nm showed the absorption maxima at 271 nm for tramadol hydrochloride and for Quercetin (Dihydrate) 372 nm. The Beer- Lambert's concentration range is for Tramadol hydrochloride 0-20 µg/mL and for Quercetin (Dihydrate) 2-20 µg/mL at respective selected wavelength. The regression coefficient for Tramadol hydrochloride and Quercetin (Dihydrate) was found to be 0.999 at 271nm and 0.999 at 372 nm respectively which indicated good correlation between concentration and absorbance within the concentration range tested. Percentage estimation of Tramadol hydrochloride 249.373± 0.296 and Quercetin (Dihydrate) in niosomal formulation was found by method is 149.685±0.144. The intra- and inter-day precision was found to be less than ± 2%

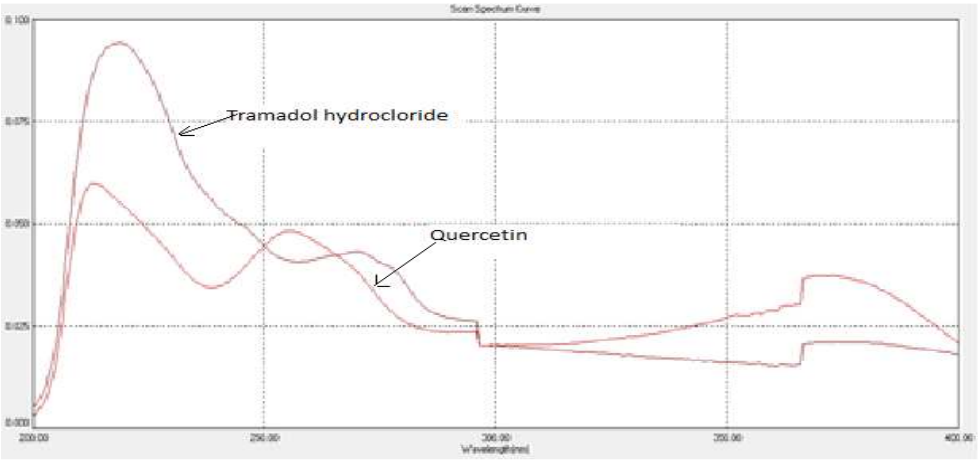

Figure 2Overlay of maximum absorption of Rutin and Quercetin (Dihydrate)

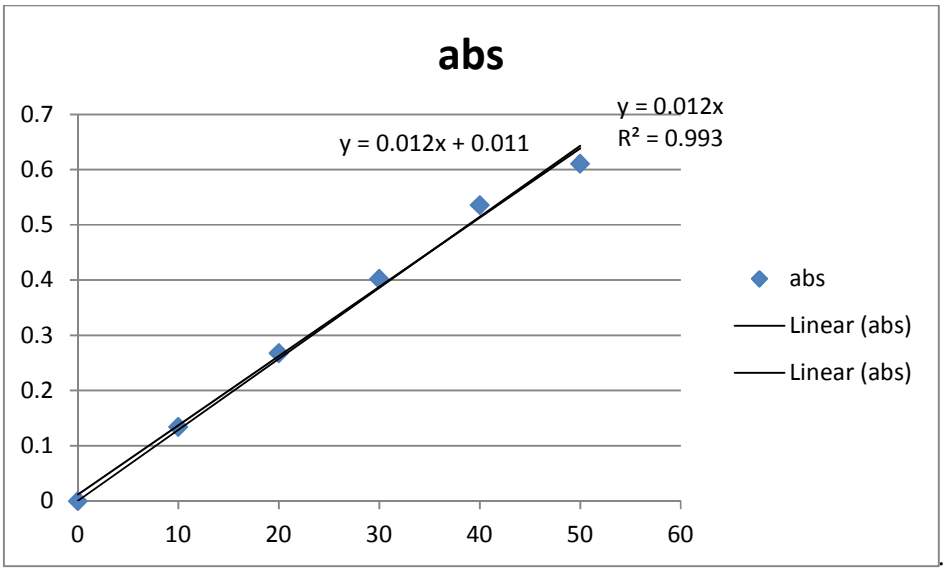

Figure 3: Calibration of tramadol HCl

Table 1: Result of validation parameter

Parameter	Tramadol HCL	Quercetin(Dihydrate)
λmax	271 nm	372 nm
Linearity range	0-20 µg/ml	2-20 µg/ml
Linearity equation	Y=0.012x+0.011	Y=0.012x+0.004
R2	0.993	0.999
Slope	0.012	0.013
LOD	0.5285 µg/ml	0.2357µg/ml
LOQ	1.6015 µg/ml	0.7142 µg/ml

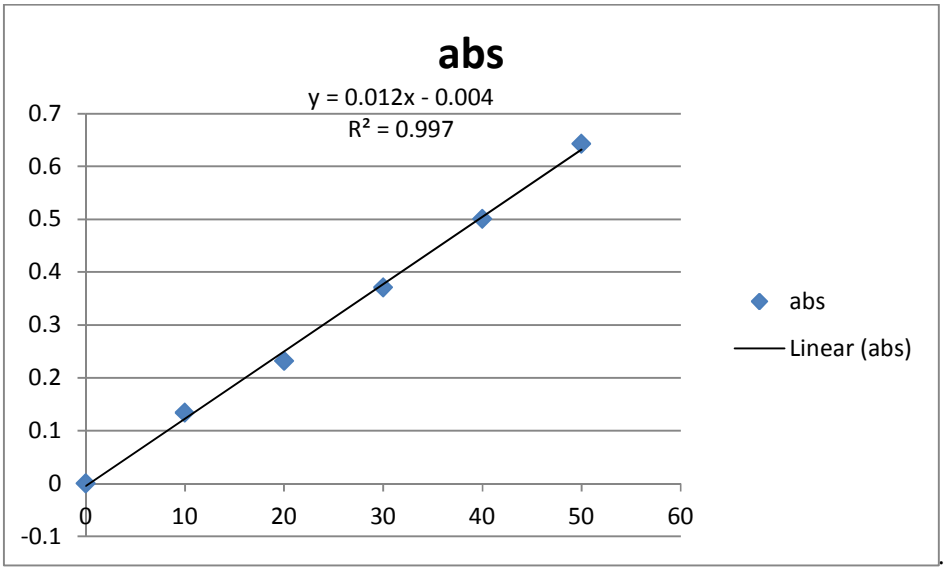

Figure 4 : Calibration of Quercetin (Dihydrate)

Table 2 : Interday and Intraday precision

	Interday precision		Intraday precision	
	%Amount found± SD*	**% RSD**	**%Amount found± SD***	**% RSD**
TRAMADOL HCL	98.38±0.7259	0.7378	97.29±0.883	0.907
QUERCETIN(DIHYDRATE)	101.96±0.7114	0.0069	102.17±1.008	0.986

Table 3: Recovery study

Concentration of the drug added to the formulation	**Tramadol HCL Recovery ±SD***	**%RSD**	Quercetin(Dihydrate) **% Recovery ± SD***	**%RSD**
80%	100.31±0.682	0.680	100.30±0.797	0.795
100%	99.86±0.3060	0.306	99.58±0.838	0.841
120%	99.88±0.1266	0.126	100.42±0.770	0.766

**Average of three determinations*

Table 4 Result of analysis of formulation

Formulation	**Drug**	**Amount Found µg/ml ± S.D***
Niosome formulation	Tramadol HCL	249.20±0.296
	Quercetin(Dihydrate)	149.68±0.114

**Average of three determinations*

Table 5 : Ruggedness study

Niosome formulation	**Drug**	**%Amount found ± S.D***
Analyst 1	Tramadol HCL	99.93± 0.914
	Quercetin(Dihydrate)	99.49±0.914
Analyst 2	Tramadol HCL	99.85± 0.524
	Quercetin(Dihydrate)	99.68± 0.554

CONCLUSION

From the results and discussion the method described in this paper for the determination of tramadol hydrochloride and Quercetin (Dihydrate) from niosomes formulation is simple, accurate, sensitive, reproducible and economical. As this proposed method utilizes inexpensive solvents and could be applied for routine analysis in quality control laboratories.

REFERENCES

[1] D. Desmeules, J. Collart[Pharmacology of tramadol] *Drugs*. 1997;53 Suppl 2:18-24. Pubmed,4/3/2015
[2] H. Sandhar, B. Kumar, S.Prasher, *Int. pharmaceuticasciencia*,**2011**,(1) 25-41
[3] S.Suzuki, T. Takada, Y. Sugawara, T. Muto, T. Kominami, *Jpn J. Cancer Res*,**1991** ,(82), 1061-1064 .
[4] D.Gharge, P.Dhabale,*Int. J. ofPharmTech Research*,**2010**,2(2)1119-1123
[5] S. Chaudhari, J. Bangar, G. Akuskar and M.Ratnaparkhi, *Der Pharmacia Lettre*, **2014**, 6 (3) 271-276
[6] R.Shukla, R. Shivkumar and K.Shivan,*Bulletin of Pharmaceutical Research* **2011**;1(1):62-6.
[6] N. Robert, A.H.Wachter, pharmaceutical process validation, third ed., pp.507-522.
[7] A. Phatak, D. Sonawane, and P.Chaudhari, *RJPBCS*, **2013**4 (3), 1269.
[8] G. Subramanian ,S. Nainar ,Y. Karthik ,*Int, J. Pharmacy and Pharm. Sci*.**2014** (6)606-608.
[9] A. H. Beckett, J. B.,Stenlake Practical pharmaceutical chemistry, CBS publications and distributors,**1997**, part ii, fourth ed., 286.
[10] K. Rajnarayana, M. Sripalreddy, M. Chaluvadi, *Ind. J. Pharmacology*.**2001**, 33,2-16.