Open Access Journal
RESEARCHERID
THOMSON REUTERS
International Journal of Medical Research and Pharmaceutical Sciences
Volume 5 (Issue 5): May 2018 ISSN: 2394-9414
DOI- 10.5281/zenodo.1256557 Impact Factor- 4.174

STABILITY INDICATING BIOANALYTICAL METHOD AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LEVOSULPIRIDE AND RABEPRAZOLE SODIUM IN HUMAN PLASMA BY RP-HPLC

SK.Ammaji[1], Sai Krishna L[2], Naresh dola[3] , Ponugupati Tarun[4] & J.S.K.Nagarajan[4]
[1]Department of Pharmaceutical chemistry University College of pharmaceutical sciences ANU, Guntur Dt, andhrapradesh.
[2]Nri college of pharmacy, agiripalli, Vijayawada, Krishna Dt, Andhra Pradesh.
[3]Department of pharmaceutical analysis Siddhartha institute of pharmaceutical sciences, narsaraopet, Guntur Dt, andhrapradesh.
[4]Department of Pharmaceutical Analysis JSS College of Pharmacy, rocklands, udhagamandalam.

Keywords:
levosulpiride, rabeprazole, amisulpiride, RP-HPLC.

Abstract

Aim: To developing, stability indicating validating bioanalytical method for the estimation of Levosulpiride and Rabeprazole Sodium by RP-HPLC method which is expected to be accurate, precise, linear, stable and selective Methods of measuring drugs in biologic media are increasingly important problems related to bioavailability and bioequivalence, new drug development, drug abuse, clinical pharmacokinetics, and drug research are highly dependent on accurately measured drugs in biological fluids.

Method: to development of N**ew Bioanalytical** method for estimation in combined oral solid formulation of Levosulpiride and Rabeprazole Sodium. Stationary phase Hibra C_{18}(250×4.6 mm i.d.,5µ) column at wavelength of 240nm. Using 10mM ammonium acetate is mobile phase in an isocratic elution mode at a flow ratio 0.8ml/min.

Result: the retention time of Levosulpiride and Rabeprazole Sodium were 9.92 and 4.27min respectively and internal standard RT was 12.04min, therefore no interference between internal standard and drug peaks. Quantitation was achieved with UV detect in at 240nm based on peak areas with linearity concentration ranges 0.024-2.4 µg/ml for levosulpiride, 0.024-1.24 µg/ml for rabeprazole sodium (R^2 value 0.9985 and 0.9993). The LOD's were 0.003µg/ml, LOQ's found to be 0.009µg/ml and 0.01µg/ml respectively.

Conclusion: the developed method for the estimation of Levosulpiride and Rabeprazole in plasma is rugged, rapid, sensitive, precise, selective and linear and is therefore, can be employed for a bioequivalence study to evaluate its applicability further.

Introduction

HPLC is a dynamic adsorption process. Analyte molecules, while moving through the porous packing beads, tend to interact with the surface adsorption sites. Depending on the HPLC mode, the different types of the adsorption forces may be included in the retention process: Hydrophobic (non-specific) interactions are the main ones in **reversed-phase (RP)** separations. Dipole-dipole (polar) interactions are dominant in **normal phase (NP)** mode. Ionic interactions are responsible for the retention in **ion-exchange** chromatography. All these interactions are competitive. Analyte molecules are competing with the eluent molecules for the adsorption sites. So, the stronger

Open Access Journal
RESEARCHERID
THOMSON REUTERS
International Journal of Medical Research and Pharmaceutical Sciences
Volume 5 (Issue 5): May 2018 ISSN: 2394-9414
DOI- 10.5281/zenodo.1256557 Impact Factor- 4.174

analyte molecules interact with the surface. The weaker the eluent interaction, the longer the analyte will be retained on the surface.

Reversed-phase chromatography is the inverse of this. The stationary bed is (nonpolar) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. To uses a non-polar hydrophobic packing with octyl or octadecyl functional group bonded to silica gel as stationary phase and an aqueous, moderately polar solvent as mobile phase. With these stationary phases, retention time is longer for molecules which are more non-polar, while polar molecules elute more readily. Reverse phase mode is the most popular mode for analytical and preparative separation of compounds of interest in chemical, biological, pharmaceutical, food and biomedical sciences. Most of the drugs in pharmaceutical are polar in nature. Being polar in nature different columns are used for the analysis of drugs such as octadecylsilane (ODS) or C_{18}, C_8, $C_{4\ etc.}$, (in the order of increasing polarity of the stationary phase).

Rabeprazole is an antiulcer drug in the class of Proton pump inhibitor it is used in the treatment of duodenal ulcer, gastroesophageal refux disease . Levosulpiride is substituted benzamide derivative under the class of antipsychotic drug. It is selective claimed to have mood elevating property particularly in treating negative symptoms of schizophrenia, anxiety, vertigo and dyspepsia. Both these drugs decrease the release of chemical mediators, block proton pump in stomach wall.

Scope of Work:
The objective of present study is Methods of measuring drugs in biologic media are increasingly important problems related to bioavailability and bioequivalence, new drug development, drug abuse, clinical pharmacokinetics, and drug research are highly dependent on accurately measured drugs in biological fluids. As per the literature review, there were analytical methods for estimation of Levosulpiride and Rabeprazole Sodium in individual and in combination but **no bio-analytical methods** were developed and reported for Levosulpiride and Rabeprazole Sodium in combined oral solid dosage formulation. For the estimation of the drugs present in the biological fluid, HPLC method is consider to be more suitable since this is a powerful and rugged method. It is also extremely specific, linear, precise, accurate, sensitive and rapid.

Materials and methods

Chemicals used
Acetonitrile of HPLC grade by Merck, Ammonium Acetate by S.D.Fine chemicals Ltd. and Water HPLC grade from Milli-Q RO system were used. Working Standard of Levosulpiride and Amisulpiride were obtained from Inchem Laboratories Pvt. Ltd., and Rabeprazole Sodium was obtained from Sigma Aldrich.

Instruments used
Shimadzu digital balance, Systronics - pH meter, µ pH system 361, Waters HPLC system with following configurations, 1515 solvent delivery system (pump), Rheodyne*7725i* injector with 20µl loop, 2487 Dual wavelength UV-VIS Absorbance detector, Breeze data station.

Shimadzu gradient HPLC system with following configurations LC-10 AT-VP solvent delivery system (pump), Rheodyne 7725*i* injector with 20 µl loop, SPD M-10AVP Photo Diode Array detector, Class-VP data station, Shimadzu (UV-1700) spectrophotometer, Ultra Sonicator, Analytical columns such as Hibar® C_{18} (250 x 4.6 mm i.d., 5 µ), PhenomenexC_{18} (250 x 4.6 mm i.d., 5 µ)

Instrument Details:
System : LC-10 AT-VP solvent delivery system
Pump : Isocratic pump

Open Access Journal
RESEARCHERID
THOMSON REUTERS
International Journal of Medical Research and Pharmaceutical Sciences
Volume 5 (Issue 5): May 2018 ISSN: 2394-9414
DOI- 10.5281/zenodo.1256557 Impact Factor- 4.174

Detector : SPD M-10AVP UV Detector
Data processor : Class VP
Injector :Rheodyne 7725 injector with 20 µl loop

Preparation of Standard Stock Solution of Rabeprazole Sodium

A standard solution of 1mg/ml of Rabeprazole Sodium was prepared by dissolving 10mg of Rabeprazole Sodiumin Acetonitrile and finally made the volume with the same solvent to 10ml. Labelled and stored the solution in a refrigerator below 8ºC.

Preparation of Standard Stock Solution of Levosulpiride
A standard solution of 1mg/ml ofLevosulpiride (10mg in 10ml) was prepared separately using Acetonitrile and finally made the volume with the same solvent. Labelled and stored the solution in a refrigerator below 8ºC.
Preparation of Standard Stock Solution of Internal standard

A standard solution of 1mg/ml of internal standard was prepared using acetonitrile and finally made the volume with the same solvent. Labelled and stored the solution in a refrigerator below 8ºC.

Preparation of Working Standard Solution:
1 ml of the Levosulpiride and Rabeprazole stock solution and 1 ml of the internal standard stock solution was diluted to 10ml in three different standard flasks with acetonitrile to achieve a concentration of 100ug/ml for each drug and internal standard. From the above solution, different working standard solutions were prepared, as per respective concentrations of solutions need by diluting with the acetonitrile.

Preparation of Rabeprazole sodium and Levosulpiride standard

Standard solution for CC
The serial dilution of 0.012, 0.024, 0.06, 0.12, 0.24, 0.49, 0.744, 0.99 and 1.24 µg/ml of Rabeprazole Sodium and 0.024, 0.048, 0.12, 0.24, 0.48, 0.96, 1.4, 1.9 and 2.4 µg/ml of Levosulpiride were prepared by spiking technique along with 200µg/ml of IS in 10ml. After analysis the solution is labeled and stored until finish the optimization.

Preparation of plasma samples by Protein Precipitation Technique
Plasma samples were prepared by taking 0.2ml of plasma, 0.2ml of drug solutions each and 0.2ml of internal standard in ria vials. These solutions are then vortexed for 15minutes and then 0.2ml of precipitating agent like acetonitrile and vortexed for 15 minutes. These solutions were then centrifuged at 3500 RPM, after centrifugation the supernatant liquid is collected made the volume to 1ml and the same will injected for the analysis.

Optimized Chromatographic conditions	
Column	**Hibra C_{18}(250×4.6 mm i.d.,5µ)**
Mobile Phase	**Ammonium acetate : acetonitrile (20:80)**
Flow rate	**0.8mL/min**
Elution mode	**Isocratic**
Detector	**PDA 240 nm**
Temperature	**Ambient**
Injection volume	**20µl**

Open Access Journal
RESEARCHERID
THOMSON REUTERS
International Journal of Medical Research and Pharmaceutical Sciences
Volume 5 (Issue 5): May 2018 ISSN: 2394-9414
DOI- 10.5281/zenodo.1256557 Impact Factor- 4.174

Bioanalytical validation of the method

Validation is a process which involves confirmation or establishment by laboratory studies that a method / procedure / system / analyst can give the required accuracy, precision, sensitivity, ruggedness, etc. In the most basic form, validation of a Bioanalytical procedure demonstrates that the procedure developed is suitable for its intended purpose. Validation of the method was carried out after the development of the HPLC method.

TABLE 1: Validation parameters:

	Levosulpiride	**Rabeprazole**
Linearity	**R^2 =0.9985**	**R^2 =0.9993**
Accuracy(%recovery)	**Plasma: 98% Mobile phase:99.5%**	**Plasma: 98.3% Mobile phase:99%**
Precision	**%RSD ≤ 0.16**	**%RSD ≤ 0.08**
LOD	**0.003(µg/ml)**	**0.003(µg/ml)**
LOQ	**0.009(µg/ml)**	**0.01(µg/ml)**

The *System suitability* parameters such as column efficiency (theoretical plates), resolution factor and peak asymmetry factor of the optimized methods were found satisfactory.

Table.2. system suitability studies

S.NO	**PARAMETERS**	**RABEPRAZOLE**	**LEVOSULPIRIDE**
1.	**Theoretical Plate**	**10101**	**13247**
2.	**Tailing factor**	**1.0**	**1.53**
3.	**Asymmetric factor**	**1.0**	**1.0**
4.	**LOD(ng/ml)**	**3.0**	**3.0**
5.	**LOQ(ng/ml)**	**10.0**	**9.0**

Estimation of levosulpiride and rabeprazole

By RP-HPLC method

Estimation of the supernatant obtained from plasma samples was carried out using the optimized chromatographic conditions. The standards were injected and chromatograms were recorded. The typical chromatograms of the Levosulpiride, Rabeprazole and Amisulpiride(IS) are given in Fig. 1& 2.

Open Access Journal
RESEARCHERID
THOMSON REUTERS
International Journal of Medical Research and Pharmaceutical Sciences
Volume 5 (Issue 5): May 2018 ISSN: 2394-9414
DOI- 10.5281/zenodo.1256557 Impact Factor- 4.174

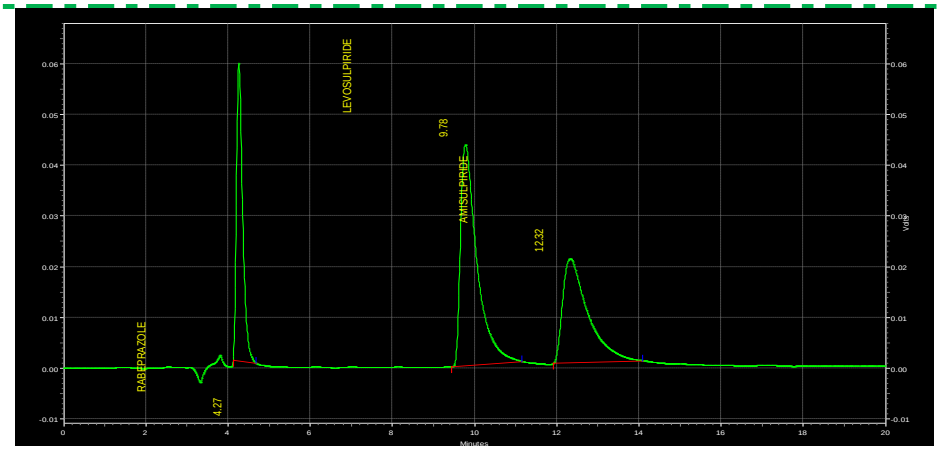

Fig 1: Typical standard chromatogram of rabeprazole, levosulpiride and amisulpiride(is)

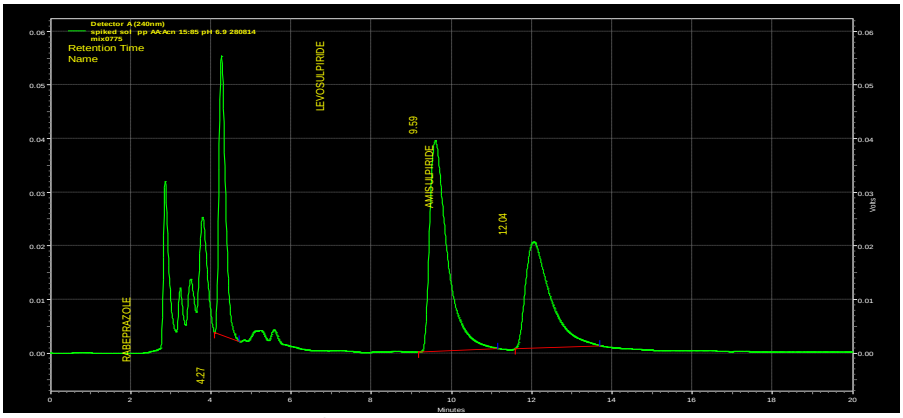

Fig 2: Typical chromatogram of rabeprazole, levosulpiride and amisulpiride(is) in plasma

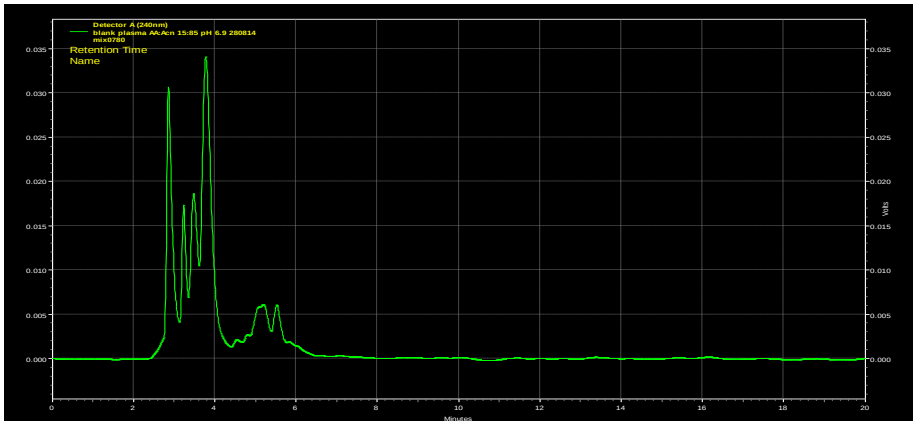

Fig 3: blank plasma

Open Access Journal
RESEARCHERID
THOMSON REUTERS
International Journal of Medical Research and Pharmaceutical Sciences
Volume 5 (Issue 5): May 2018 ISSN: 2394-9414
DOI- 10.5281/zenodo.1256557 Impact Factor- 4.174

The calibration curves were constructed routinely for spiked plasma containing Levosulpiride and Rabeprazole and internal standard during process of pre-study validation and in-study validation.

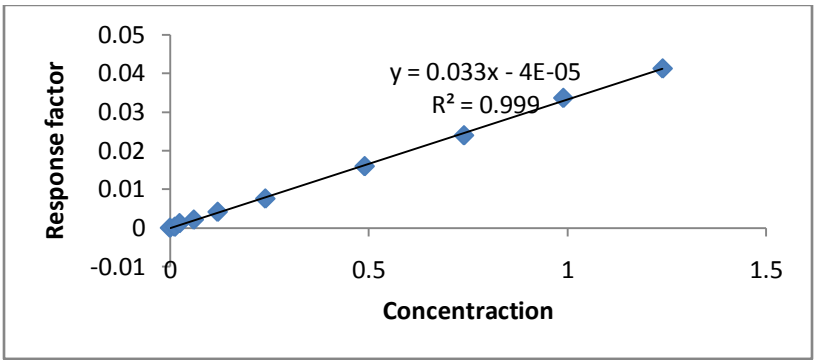

Fig 4: calibration curve of rabeprazole

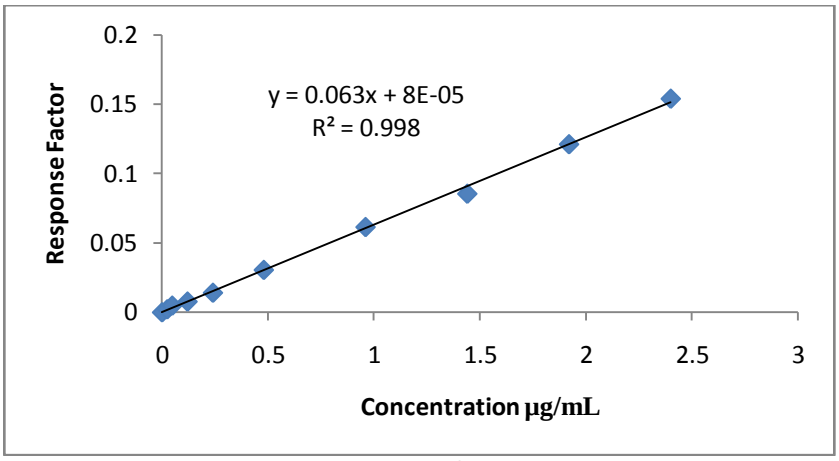

Fig 5: calibration curve of levosulpiride

The mobile phase used for the estimation provided a well-defined separation between the drugs, internal standard and endogenous components. The blank plasma showed no interference at the retention time of both the Levosulpiride and Rabeprazole and internal standard.

Stabilitystudies

Stability of the sample, standard and reagents used in a HPLC method is required for a reasonable time to generate reproducible and reliable results. Stability of plasma samples spiked with drugs were subjected to three freeze-thaw cycles, short term stability at ambient temperature for 3 hours and long term stability at –70° C for two weeks**.** In addition, stability of standard solutions and internal standards were performed at ambient temperature for 6 hours and under frozen condition for six days. The stability of these solutions was studied by performing the experiment and looking for changes in separation, retention and asymmetry of the peaks which were then compared with the pattern of the chromatogram of freshly prepared solutions. The values have been recorded in the following table 3.

Open Access Journal
RESEARCHERID
THOMSON REUTERS
International Journal of Medical Research and Pharmaceutical Sciences
Volume 5 (Issue 5): May 2018 ISSN: 2394-9414
DOI- 10.5281/zenodo.1256557 Impact Factor- 4.174

Table.3. stability of *rabeprazole*in plasma during storage and sample handling
Short Term Stability data **(at Ambienttemperature)**

Nominal Concentration (µg/ml)			
Short Term Plasma at Ambient Temperature	**LQC**	**MQC**	**HQC**
	0.06	**0.49**	**0.99**
After 1 hr	0.0581	0.478	0.9840
After 2 hrs	0.0581	0.478	0.9840
After 3 hrs	0.0582	0.476	0.9841
Mean	**0.0581**	**0.4773**	**0.984**
S.D (+/-)	**0.00005**	**0.00115**	**0.00005**
C.V. (%)	**0.10**	**0.24**	**0.01**
% Nominal	**96.83**	**97.40**	**99.39**
N	**3**	**3**	**3**

Table.3. stability of rabeprazolein plasma during storage and sample handling
Long Term Stability data **(at -70°C)**

Nominal Concentration (µg/ml)			
Plasma stored at -70°	**LQC**	**MQC**	**HQC**
	0.06	**0.49**	**0.99**
After 1 week	0.0581	0.477	0.9840
After 2 weeks	0.0577	0.474	0.9838
Mean	0.0579	0.4755	0.9839
S.D (+/-)	**0.00028**	**0.0021**	**0.00014**
C.V. (%)	**0.49**	**0.45**	**0.01**
% Nominal	**96.5**	**97.04**	**99.38**
N	**3**	**3**	**3**

Open Access Journal
RESEARCHERID
THOMSON REUTERS
International Journal of Medical Research and Pharmaceutical Sciences
Volume 5 (Issue 5): May 2018 ISSN: 2394-9414
DOI- 10.5281/zenodo.1256557 Impact Factor- 4.174

Table:3. stability of rabeprazolein plasma during storage and sample handling

Freeze and Thaw Stability data

Nominal Concentration (µg/ml)			
	LQC	**MQC**	**HQC**
	0.06	**0.49**	**0.99**
Cycle 1	0.0581	0.477	0.9841
Cycle 2	0.0581	0.478	0.9842
Cycle 3	0.0580	0.477	0.9842
Mean	**0.058**	**0.477**	**0.9841**
S.D (+/-)	**0.00005**	**0.00057**	**0.00005**
C.V. (%)	**0.1**	**0.12**	**0.01**
% Nominal	**96.66**	**97.34**	**99.39**
N	**3**	**3**	**3**

Table:4 Stability of levosulpiride in plasma duringstorage and sample handling

Short Term Stability data **(at Ambienttemperature)**

Nominal Concentration (µg/ml)			
Short Term Plasma at Ambient Temperature	**LQC**	**MQC**	**HQC**
	0.12	**0.96**	**1.9**
After 1 hr	0.1181	0.9552	1.880
After 2 hrs	0.1180	0.9552	1.882
After 3 hrs	0.1179	0.9550	1.879
Mean	**0.118**	**0.9551**	**1.880**
S.D (+/-)	**0.0001**	**0.00011**	**0.0015**
C.V. (%)	**0.08**	**0.01**	**0.08**
% Nominal	**98.33%**	**99.48%**	**98.94%**
N	**3**	**3**	**3**

Open Access Journal
RESEARCHERID
THOMSON REUTERS
International Journal of Medical Research and Pharmaceutical Sciences
Volume 5 (Issue 5): May 2018 ISSN: 2394-9414
DOI- 10.5281/zenodo.1256557 Impact Factor- 4.174

Table.4 (continues). stability of levosulpiride in plasma duringstorage and sample handling

***Long Term Stability data* (at -70°C)**

Nominal Concentration (µg/ml)			
Long Term Plasma Sample at -70°	**LQC**	**MQC**	**HQC**
	0.12	**0.96**	**1.9**
After 1 week	0.1178	0.9551	1.880
After 2 weeks	0.1179	0.9550	1.878
Mean	**0.1179**	**0.9551**	**1.879**
S.D (+/-)	**0.00007**	**0.00007**	**0.00141**
C.V. (%)	**0.06**	**0.01**	**0.08**
% Nominal	**98.25**	**99.48**	**98.89**
N	**3**	**3**	**3**

Table:4(continues). stability of levosulpiridein plasma duringstorage and sample handling

Freeze and Thaw stability

Nominal Concentration (µg/ml)			
	LQC	**MQC**	**HQC**
	0.12	**0.96**	**1.9**
Cycle 1	0.1180	0.9550	1.881
Cycle 2	0.1181	0.9551	1.880
Cycle 3	0.1180	0.9550	1.882
Mean	**0.1180**	**0.955**	**1.881**
S.D (+/-)	**0.00005**	**0.00005**	**0.001**
C.V. (%)	**0.05**	**0.01**	**0.05**
% Nominal	**98.33**	**99.4**	**99.0**
N	**3**	**3**	**3**

Open Access Journal
RESEARCHERID
THOMSON REUTERS
International Journal of Medical Research and Pharmaceutical Sciences
Volume 5 (Issue 5): May 2018 ISSN: 2394-9414
DOI- 10.5281/zenodo.1256557 Impact Factor- 4.174

Conclusion

The purpose of the present study was to develop and validate a Bio-analytical method by RP-HPLC for Simultaneous Estimation of Rabeprazole and Levosulpiride in Human Plasma. The Current method was developed and Validated for the estimation of combination of the above drugs in **human plasma over concentration range of 0.012 to 1.24 µg/ml of Rabeprazoleand 0.024to 2.4 µg/ml of Levosulpiride.** The developed bioanalytical method is most convenient method of analysis by employing simple solvent system with high resolution, shorter analysis time for the estimation of Levosulpiride and Rabeprazole. The method is linear, precise, accurate, robust as per acceptance criteria so it can be employed for a biopharmaceutical, bioequivalence and clinical study to evaluate its applicability further.

References

1. *Modern analytical chemistry,davidHarvey,depauw university.*
2. *DouglesA. Skog, analytical chemistry,7th edition, saunders college publishers philadelphia, 1996, pg1-15.*
3. *Robert D.Braun,Introduction to instrumental analysis,1stEdn,Mcgraw hill book company,1987, pg1-13.*
4. *W.W.Brain and Dreck,samples and standards, analytical chemistry by open learning ,john wiley and sons,London 1991, pg2-5.*
5. *jamesW.robinson,under graduate instrumental analysis,5thedition marcel dekker,new York 1997, pg584-589.*
6. *sandyLindsay,HPLC by open learning,Johnwiley and sons 1991, pg30-45.*
7. *david G Watson pharmaceutical analysis Churchill Livingston reprinted 2000.*
8. *hand book of instrumental techniques for analytical chemistry by frank settle,HPLC-MS chhabilDass 1997, pg647-664.*
9. *LIoydR.Snyder , joseph J Kirkland and joseph L.Glajch,practicalHPLCMethod development 2ndedition 1997, pg1-14*
10. *Validation of A nalyticalprocedures :Methodology,ICH harmonised Tripartite gidelines 1996, pg1-8.*
11. *Guidance for industry "bio analytical method validation " http://www.fda.gov/cder/guidence/index.htm may 2001; pg1-25*
12. *Shobamanjunath,venkateshchouhanand Sandeep.,spectrophotometric estimation of levsulpiride in bulk drug formulation Int J Pharma science ,vol 3,issue 2,2011, pg135-137.*
13. *ChhalotiyaUsmaganiK,BhattKashyap K, Shah DimalA,BaldaniasunilL,PatelJiger R, Development of stability indicating RP-HPLC Method for determination of LevoSulpiride HCL bulk and Pharmaceutical dosage form,IJAPA vol2,Issue 2,2012, pg41-46*
14. *Bijaykumarsahoo,AyaandasjayanthiMukherjee,SoumendraDrbar and Tapankumarpal,determination of levosulpiride in human plasma using HPLC Method and its application to bio equivalence study http://dx.doi.org/10.4172/scienticrepots.523.*
15. *Jin E, ban E etal., journal of pharma biomedical analysis, jan29, 2014, 35(4), pg no 929-36.*
16. *S.elumali, kiran aher etal., journal of applied pharmaceutical ciences, 01(06), 2011, pg no-165-170.*
17. *Kumar N, Sangeetha D et al., science pharma, march 17, 81(3), 2013, pg no 697-711.*
18. *Yoganad B, Deulgaonkar et al., indo American journal of pharmaceutical research vol.3, issue 5, 2013, pg no 4017-4025.*
19. *Nandakoshor agarwal, B.jagadesh, international journal of pharma& biosciences, oct 3(4), 2012, pg no 718-726.*
20. *A.sirisha, A.Ravi kumar, international research journal of pharmaceutical and applied sciences, 2(4), 2012, pg no 49-55.*