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A new RP-HPLC method for the simultaneous estimation of flupentixol and melitracen in its pure and pharmaceutical dosage form as per ICH guidelines

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ABSTRACT

A precise, simple, accurate and selective method was developed and validate for simultaneous estimation of Flupentixol and Melitracen in API form and Pharmaceutical Dosage Form. Reversed phase high performance liquid chromatographic (RP-HPLC) method was developed for routine quantification of Flupentixol and Melitracen in the API form as well as in combined pharmaceutical dosage form. Chromatographic separation was achieved on a Phenomenex Gemini C18 (4.6×250mm) 5µm particle size utilizing mobile phase of filtered and degassed mixture of Methanol and Phosphate buffer(pH-3.8) (40:60% v/v) at a flow rate of 1 mL/min with UV detection at 225 nm. The method has been validated for linearity, accuracy and precision. In RP-HPLC method, the calibration graphs were linear in the concentration range of 10-30 µg/ml for Flupentixol and 30-90µg/ml for Melitracen with percentage recoveries are within the limits. The results obtained by RP-HPLC methods are rapid, accurate and precise. Therefore proposed method can be used for routine analysis of Flupentixol and Melitracen in the API form as well as in combined pharmaceutical dosage form.

Keywords: Flupentixol and Melitracen, RP-HPLC, Validation, ICH Guidelines.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components.[1] Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new

toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. [1, 15]

DIFFERENT METHODS OF ANALYSIS

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample. E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.2

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture. E.g. Potentiometry, Conductometry, Amperometry.[2]

Chromatographic methods

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics. E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC). [2]

Miscellaneous Techniques

Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques

GC-MS (Gas Chromatography–Mass Spectrometry), LC-MS (Liquid Chromatography–Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography–Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry). Analytical techniques that are generally used for drug analysis also include biological and microbiological methods, radioactive methods and physical methods etc. are mentioned in Table.[2]

Table 1: Summary of Hyphenated separation techniques.[2]

Separation technique	Hyphenated mode
Liquid chromatography	Liquid chromatography-mass spectrometry(LC/MS) Liquid chromatography-Fourier-transform infrared Spectrometry(LC-FTIR) Liquid chromatography-nuclear magnetic resonance spectroscopy (LC/NMR) Liquid chromatography-inductively coupled plasma mass spectrometry (LC-ICPMS)
Gas chromatography	Gas chromatography-mass spectrometry(GC/MS) Gas chromatography-Fourier-transform infrared(GC-FTIR) Gas chromatography-FTIR-MS(GC-FTIR-MS)
Capillary electrophoresis	Capillary electrophoresis-mass spectrometry(CE/MS) Capillary electrophoresis- nuclear magnetic resonance spectroscopy(CE/NMR) Capillary electrophoresis-surface enhanced Raman spectrometry (TLC-SERS)
Thin layer chromatography(TLC)	Thin layer chromatography- mass spectrometry(TLC/MS) Thin layer chromatography- surface enhanced Raman spectrometry(TLC-SERS)

Superficial fluid chromatography/ extraction(SFC/SFE)	Superficial fluid extraction-capillary gas chromatography-mass spectrometry(SFE-CGC-MS) Superficial fluid-Fourier-transform infrared(SFC-FTIR)

MATERIALS AND METHODS

Flupentixol (Pure) from Sura labs, Melitracen (Pure) from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck, Phosphate buffer from Sura labs.

Hplc method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Flupentixol and Melitracen working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.2ml of Flupentixol and 0.6ml of Melitracen from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol. Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: WaterandACN: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Phosphate buffer in proportion 40:60 v/v respectively.

Optimization of Column

The method was performed with various C18columns like Symmetry, X terra and ODS column. Phenomenex Gemini C18 (4.6×250mm) 5µm was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions

Instrument used : Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature : 35ºC
Column : Phenomenex Gemini C18 (4.6×250mm) 5µm particle size
Mobile phase : Methanol and Phosphate buffer(pH-3.8) (40:60% v/v)
Flow rate : 1ml/min
Wavelength : 225nm
Injection volume : 20µl
Run time : 6minutes

Validation

Preparation of mobile phase

Preparation of mobile phase

Accurately measured 400ml of Methanol (40%) of and 600ml of HPLC Water (60%) were mixed and degassed in adigital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio: Methanol and Phosphate buffer (pH-3.8) (40:60% v/v)
Column : Phenomenex Gemini C18 (4.6×250mm) 5µm particle size
Column temperature: 35ºC
Wavelength : 225nm
Flow rate : 1ml/min
Injection volume : 20µl
Run time : 6minutes

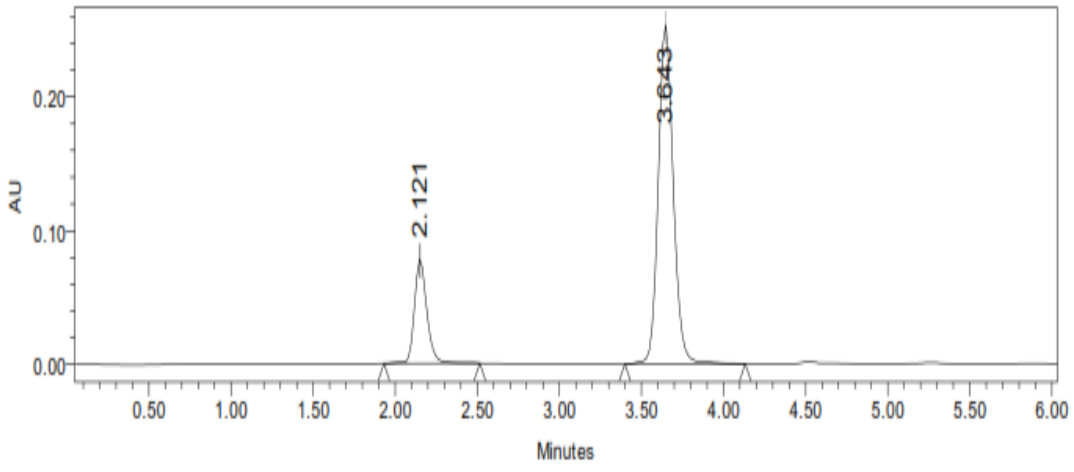

Fig 1: Optimized Chromatogram (Standard)

Optimized Chromatogram (Sample)

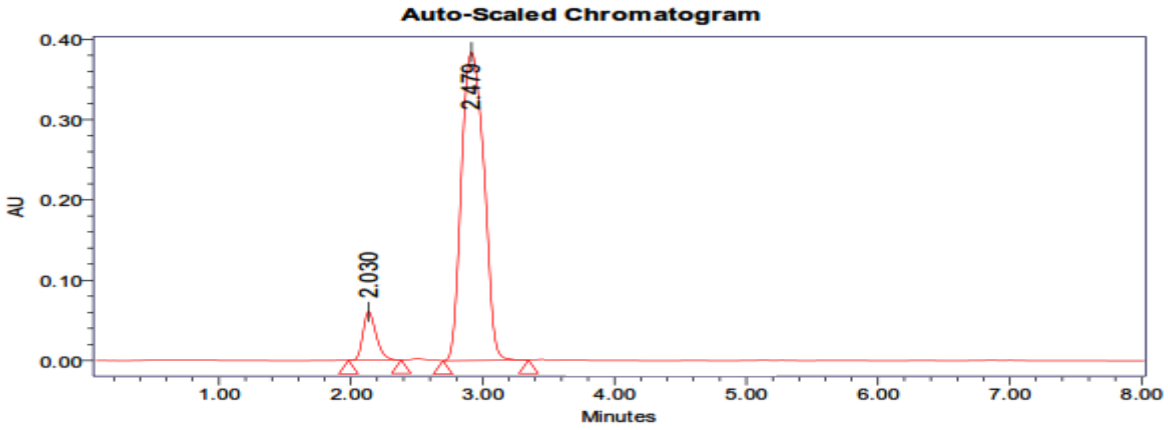

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.no	Name	Rt	Area	Height	USPTailing	USPPlate Count	Resolution
1	Flupentixol	2.142	512659	78956	1.2	4652	
2	Melitracen	3.649	1615985	263587	1.1	7982	10.3

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay (Standard)

Table 3: Results of system suitability for Flupentixol

S.No			Area (µV*sec)	Height (µV)		
1	Flupentixol	2.152	513652	78542	4698	1.2
2	Flupentixol	2.157	513524	78654	4785	1.2
3	Flupentixol	2.141	513425	78541	4682	1.2
4	Flupentixol	2.133	513647	78454	4854	1.2
5	Flupentixol	2.166	514824	78655	4872	1.2
Mean			513814.4			
Std.Dev.			572.2004			
%RSD			0.111363			

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Table 4: Results of system suitability for Melitracen

S.No			Area	Height			Resolution
1	Melitracen	3.674	1635285	265421	7985	1.1	10.1
2	Melitracen	3.631	1635241	265484	7898	1.1	10.1
3	Melitracen	3.625	1652547	253498	7954	1.1	10.1
4	Melitracen	3.692	1658458	265241	7965	1.1	10.1
5	Melitracen	3.629	1652894	265348	7985	1.1	10.1
Mean			1646885				
Std.Dev.			10865.58				
%RSD			0.659766				

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable

Table 5: Peak results for Assay sample of Flupentixol

S.No	Name	RT	Area	Height	USPTailing	USP Plate Count	Injection
1	Flupentixol	3.651	513265	78548	1.2	4582	1
2	Flupentixol	2.150	513254	78547	1.2	4658	2
3	Flupentixol	2.187	513876	78498	1.2	4597	3

Table 6: Peak results for Assay sample of Melitracen

S.No	Name	RT	Area	Height	USPTailing	USPPlateCount	Injection
1	Melitracen	3.646	1625284	78569	1.1	7985	1
2	Melitracen	3.651	1624613	78547	1.1	7898	2
3	Melitracen	3.601	1625874	78462	1.1	7854	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

=1605195 /1604865 ×10/198×198/0.3043×99.5/100×0.7/23×100 = 99.7%
The % purity of Flupentixol andMelitracen in pharmaceutical dosage form was found to be 99.7%

Linearity

Chromatographic data for linearity study of flupentixol

Concentration µg/ml	Average Peak Area
10	245899
15	365687
20	481526
25	589854
30	705882

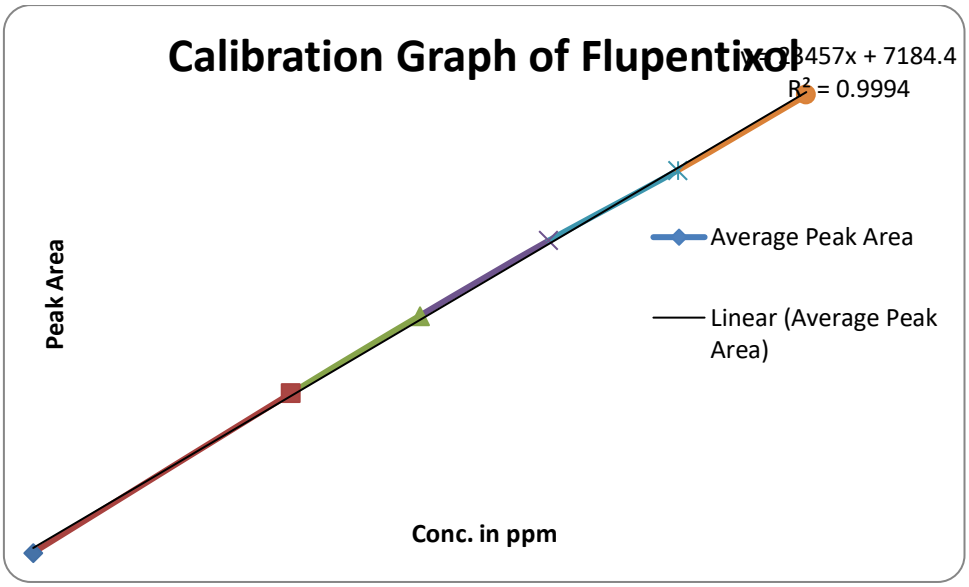

Fig 3: Calibration Graph of Flupentixol

Chromatographic data for linearity study of melitracen

Concentration µg/ml	Average Peak Area
30	863094
45	1249397
60	1678592
75	2050412
90	2468444

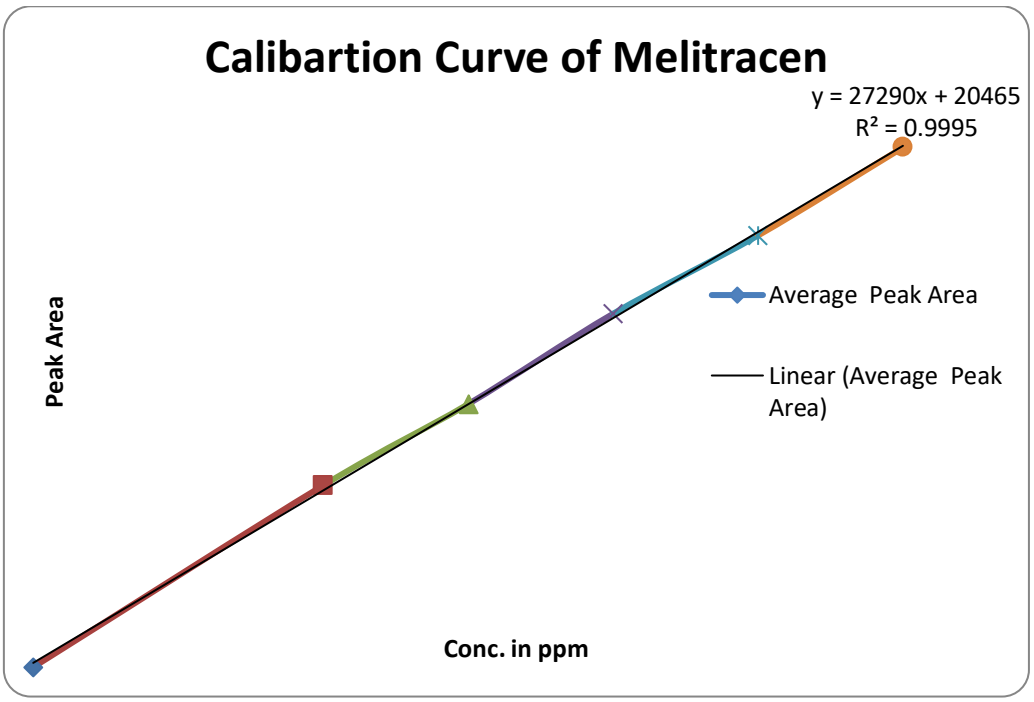

Fig 4: Calibration Curve of Melitracen

Repeatability

Table 7: Results of repeatability for Flupentixol

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Flupentixol	2.157	513568	78546	1.2	4528
2	Flupentixol	2.159	513685	78541	1.2	4572
3	Flupentixol	2.186	513659	79852	1.2	4598
4	Flupentixol	2.160	513254	78498	1.3	4529
5	Flupentixol	2.170	513647	77898	1.2	4572
Mean			513562.6			
Std.dev			177.9475			
%RSD			0.03465			

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 8: Results of repeatability for Melitracen

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Melitracen	3.603	1635625	265325	1.1	7985
2	Melitracen	3.608	1658744	264588	1.1	7859
3	Melitracen	3.600	1652985	265985	1.2	7845
4	Melitracen	3.696	1645898	264898	1.1	7969
5	Melitracen	3.629	1652364	268489	1.1	7846
Mean			1649123			
Std.dev			8811.631			
%RSD			0.534322			

Intermediate precisio

Table 9: Results of Intermediate precisionDay 1 for Flupentixol

S.No			Area	Height		
1	Flupentixol	2.198	514658	78698	4658	1.2

2	Flupentixol	2.196	514354	78599	4598	1.2
3	Flupentixol	2.160	513985	79854	4652	1.2
4	Flupentixol	2.160	514875	79879	4561	1.2
5	Flupentixol	2.160	514658	79865	4659	1.2
6	Flupentixol	2.186	516452	79854	4589	1.2
Mean			514830.3			
Std.Dev.			852.3705			
%RSD			0.165563			

- %RSD of five different sample solutions should not more than 2.

Table 10: Results of Intermediate precisionDay 1 for Melitracen

S.No			Area	Height (µV)			Resolution
1	Melitracen	3.623	1645875	266589	7985	1.1	10.1
2	Melitracen	3.611	1658554	265898	8001	1.1	10.1
3	Melitracen	3.696	1649854	265415	7985	1.1	10.1
4	Melitracen	3.696	1659842	265154	7956	1.1	10.1
5	Melitracen	3.696	1645985	266598	7985	1.1	10.1
6	Melitracen	3.642	1659852	265341	8002	1.1	10.1
Mean			1653327				
Std.Dev.			6838.733				
%RSD			0.413635				

- %RSD of five different sample solutions should not more than 2.

Table 11: Results of Intermediate precision Day 2 for Flupentixol

S.No			Area	Height		
1	Flupentixol	2.198	514658	78572	4672	1.2
2	Flupentixol	2.196	514895	78516	4639	1.2
3	Flupentixol	2.178	514658	78572	4783	1.2
4	Flupentixol	2.142	514784	78372	4623	1.2
5	Flupentixol	2.177	515268	78592	4639	1.2
6	Flupentixol	2.177	514598	78526	4737	1.2
Mean			514810.2			
Std.Dev.			248.5224			
%RSD			0.048275			

- %RSD of five different sample solutions should not more than 2.

Table 12: Results of Intermediate precision Day 2 for Melitracen

S.No			Area	Height			Resolution
1	Melitracen	3.611	1638732	264384	7985	1.1	10.1
2	Melitracen	3.623	1637438	265827	7946	1.1	10.1
3	Melitracen	3.684	1638474	266382	7943	1.1	10.1
4	Melitracen	3.697	1634273	269183	7964	1.1	10.1
5	Melitracen	3.684	1636372	261931	7968	1.1	10.1
6	Melitracen	3.684	1639283	264356	7982	1.1	10.1
Mean			1637429				
Std.Dev.			1860.366				
%RSD			0.113615				

%RSD of five different sample solutions should not more than 2

Accuracy

Table 13: The accuracy results for Flupentixol

%Concentration	Area	Amount	Amount	% Recovery	Mean
50%	245954	10	10.179	101.79%	101.36%
100%	483747	20	20.316	101.58%	

Table 14: The accuracy results for Melitracen

%Concentration	Area	Amount	Amount	% Recovery	Mean
50%	842287	30	30.114	100.38%	

100%	1659744	60	60.068	100.113%	100.26%
150%	2483885	90	90.268	100.297%	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 15: Results for Robustness

Flupentixol

Parameter used for sample	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 1.0 mL/min	513567	2.121	4536	1.2
Less Flow rate of 0.9 mL/min	523652	2.210	4462.3	0.9
More Flow rate of 1.1 mL/min	502146	2.184	4325.1	1.0
Less organic phase	521574	2.200	4632.4	0.9
More Organic phase	502416	2.172	4190.8	0.8

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

MELITRACEN

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 1.0 mL/min	1625892	3.643	4536	1.1
Less Flow rate of 0.9 mL/min	1758455	4.498	4426.4	0.9
More Flow rate of 1.1 mL/min	1742514	3.505	4421.5	0.8
Less organic phase	1726451	4.504	4355.1	0.9
More organic phase	1725466	3.512	4426.6	0.9

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Flupentixol and Melitracen in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Flupentixol was found to be freely soluble in water, Soluble in Acetone, Dimethyl sulfoxide, Ethanol, 0,1N HCl, very soluble in methanoland Melitracen was found to be Soluble in dilute ammonia, or sodium hydroxide; also soluble in methanol, Slightly soluble in water, freely soluble in sodium hydroxide solution, in n-butyl amine, and in dimethylformamide; sparingly soluble in methanol; insoluble in ether, in chloroform, and in dilute mineral acids. Methanol and Phosphate buffer(pH-3.8) (40:60% v/v)was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed inTablesfor RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Flupentixol and Melitracen in bulk drug and in Pharmaceutical dosage forms.

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