ISSN- 2394-5125 VOL 07, ISSUE 07, 2020

Development of stability indicating HPTLC method for simultaneous estimation of flupentixol (FLUP) and melitracen (MELI) in pharmaceutical preparations

Anup Barsagade*, Akshay Meshram and Swati Patil
***Dr. Anup G. Barsagade**
Assistant Professor
Maharashtra Institute of Pharmacy,
Gondwana University, Gadchiroli
Armori Road, Bramhapuri – 441206, Maharashtra, INDIA.
E-Mail: agbarsagade@gmail.com
Phone: +918355906946

Summary

The objective of current study was to develop a validated, specific stability indicating normal-phase high performance thin layer chromatographic method for simultaneous estimation of flupentixol and melitracen in their combined dosage form. The forced degradation studies were performed on pure flupentixol and melitracen and also on their combined dosage form using acid, base, neutral, oxidation, thermal and photo stress to show the stability indicating capability of the developed method. The chromatographic method was optimized using samples generated in forced degradation studies. Good separation between the peaks corresponding to the flupentixol and melitracen and degradation products from the analyte were achieved on silica gel $60F_{254}$TLC plate using toluene: chloroform: methanol: ammonia (4.5: 4.5: 1: 0.3 v/v) as mobile phase. Densitometric quantification was performed at 295 nm by reflectance scanning. The R_F value of flupentixol and melitracen were 0.45 ± 0.03 and 0.65 ± 0.03 respectively. Validation of the developed method was conducted as per ICH requirements. Response were a linear function of concentration of flupentixol over the range 50-250 ng/band by peak area with correlation coefficient 0.98174 and losartan over the range 1000–5000 ng/band by peak area with correlation coefficient 0.99107. LOD values were found to be 2.95 and 5.48 ng/band by area for flupentixol and melitracen respectively. LOQ values were 8.95 and 16.62 ng/band by area for flupentixol and melitracen respectively. Results from analysis of a commercial tablet formulation were 100.38 ± 0.1959 % and 100.03 ± 0.3214 % by peak area for flupentixol and melitracen respectively. Recoveries were 99.16 ± 0.8177 % and 99.63 ± 0.9331 % by peak area for flupentixol and melitracen respectively. The conditions used also enabled separation and detection of degradation products from acidic, basic, oxidation stress. No degradation products of FLUP and MELI were obtained after neutral, photo and heat stress conditions applied on both drugs individually or in combination

Key Words:

HPTLC, Flupentixol, Melitracen, Degradant, Validation

ISSN- 2394-5125 VOL 07, ISSUE 07, 2020

Introduction

Flupentixol (FLUP) (EZ)-2-[4-[3-[2-(trifluoromethyl)thioxanthen-9-ylidene] propyl]piperazin-1-yl]ethanol [Figure 1] is antipsychotic drug used in the treatment of schizophrenia and in low doses use as antidipressent. Melitracen (MELI3-(10, 10-dimethylanthracen-9(10*H*)-ylidene)-N, N-dimethylpropan-1-amine [Figure 2] is a tricyclic anti-depressant used in the treatment of anxiety and depression. Literature survey revealed estimation of flupentixol and melitracen by UV Spectroscopy in alone [1-2] and in combination with other drug [3-6], HPLC in combination with other drug [7-9] and HPTLC in combination with other drugs [9-10] has been reported.

In this manuscript we describe a simple, specific, rapid, precise and accurate stability-indicating HPTLC method which is useful for analysis of FLUP and MELI and its degradation products in pharmaceutical preparations on the basis of peak area.

Experimental

Chemicals, Reagents and Solutions

Pharmaceutical grade Flupentixol and Melitracen were procured as a gift samples from Unichem Laboratories, Goa. FLUTHIXOLE a tablet formulation, obtained commercially.

Toluene, methanol, chloroform, ammonia, hydrochloric acid, sodium hydroxide and hydrogen peroxide 30% of analytical grade were used throughout the work.

To prepare standard solution of FLUP, 5 mg was accurately weighed and dissolved in small amount of methanol and the volume was made up to 10 ml with the same solvent (conc.: 0.5 mg/ml). Two milliliter of the resulting solution was then diluted to 10 ml to furnish a solution of concentration 100 µg/ml of FLUP. Two milliliter of the resulting solution was then diluted to 10 ml to furnish a solution of concentration 20 µg/ml of FLUP

To prepare standard solution of MELI, 20 mg was accurately weighed and dissolved in small amount of methanol and the volume was made up to 10 ml with the same solvent (conc.: 2 mg/ml). Two milliliter of resulting solution was diluted to 10 ml with methanol to furnish a solution of concentration 400 µg/ml of MELI.

Forced Degradation of Flupentixol and Melitracen (Stress Studies)

Stress studies were performed to determine the effect of a wide range of pH, heat, oxidizing, and photolytic conditions on FLUP and MELI. FLUP (5 mg) was weighed into 10 mL volumetric flasks and dissolved in 10 mL 1 M aqueous hydrochloric acid, For alkaline hydrolysis, FLUP (10 mg) was weighed in round bottom flask and dissolved in 20 mL 1 M aqueous sodium hydroxide and were kept at 80°C. For neutral degradation, 10 mg of FLUP was weighed in round bottom flask and dissolve in 20 ml of distilled water. The solutions were heated under reflux on a water bath at 80°C. For oxidative degradation, 5 mg FLUP was dissolved in 10 mL 3 % H_2O_2 (0.5 mg mL^{-1}) in volumetric flask and kept in dark at room temperature. For photo degradation, FLUP was evenly spread in a thin layer in a covered Petri dish and exposed to sunlight. The same amount of sample was placed in a Petri dish for thermal degradation at 70 °C.

ISSN- 2394-5125 VOL 07, ISSUE 07, 2020

Similarly, MELI (10 mg) was weighed into 10 mL volumetric flasks and dissolved in 10 mL 1 M aqueous hydrochloric acid, For alkaline hydrolysis, MELI (20 mg) was weighed in round bottom flask and dissolved in 20 mL 1 M aqueous sodium hydroxide and were kept at 80°C. For neutral degradation, 20 mg of MELI was weighed in round bottom flask and dissolve in 20 ml of distilled water. The solutions were heated under reflux on a water bath at 80°C. For oxidative degradation, 10 mg MELI was suspended in 10 mL 3% H_2O_2 (1 mg mL^{-1}) in volumetric flask and kept in dark at room temperature. For photo degradation, MELI was evenly spread in a thin layer in a covered Petri dish and exposed to sunlight. The same amount of sample was placed in a Petri dish for thermal degradation at 70 °C.

For drug-drug interaction study FLUP (0.5 mg) and MELI (10 mg) were weighed, mix and transfer into 10 mL volumetric flasks. FLUP (0.5 mg) and MELI (10 mg) were weighed into 10 mL volumetric flasks and dissolved in 10 mL 1 M aqueous hydrochloric acid, For alkaline hydrolysis, FLUP (0.5 mg) and MELI (10 mg) were weighed in round bottom flask and dissolved in 10 mL 1 M aqueous sodium hydroxide and were kept at 80°C. For neutral degradation, FLUP (0.5 mg) and MELI (10 mg) were weighed in round bottom flask and dissolve in 10 ml of distilled water. The solutions were heated under reflux on a water bath at 80°C. For oxidative degradation, FLUP (0.5 mg) and MELI (10 mg) were suspended in 10 mL 3% H_2O_2 in volumetric flask and kept in dark at room temperature. For photo degradation, FLUP and MELI were mixed and evenly spread in a thin layer in a covered petri dish and exposed to sunlight. The same amount of sample mixture was placed in a petri dish for thermal degradation at 70 °C.

Chromatography

Chromatography was performed on 10 cm × 20 cm HPTLC plates coated with silica gel 60 F_{254}. Before use plates were washed with AR-grade methanol and activated at 115°C for 30 min. Samples (5 µL) were applied to the plates as bands 5 mm wide and 3 mm apart by use of a CAMAG Linomat IV automatic sample applicator equipped with a Hamilton syringe. The application rate was 5 s μL^{-1}.

solvents firstly individual with varying polarity and their mixtures were tried for adequate separation of FLUP and MELI from each other as well as from their degradation products (DPs). Finally system containing mixture of toluene: chloroform: methanol: ammonia (4.5: 4.5: 1: 0.3 v/v) was found to be adequate for doing the same. R_F of FLUP about 0.45 ± 0.03 and R_F of MELI about 0.65 ± 0.03 (Fig. 4.9.1).

Initially, pure drugs solution was chromatographed using single solvents to ascertain the movement of the drug. Use of toluene: chloroform: methanol: ammonia (4.5: 4.5: 1: 0.3 v/v) as mobile phase gives well separated peaks of drugs and separation of degradation products from drugs as well. The R_F of FLUP about 0.45 ± 0.03 and R_F of MELI about 0.65 ± 0.03 respectively. Typical HPTLC densitogram (295 nm) was obtained from standard solution is shown in *Fig. 3.*

Samples obtained from forced degradation were chromatographed with the optimized mobile phase and it was found that densitogram obtained after acidic hydrolysis gave

ISSN- 2394-5125 VOL 07, ISSUE 07, 2020

degradation product of MELI at R_F value 0.53 ± 0.03 (MDP-I), alkaline hydrolysis gave degradation product of MELI at R_F values 0.58 ± 0.03 (MDP-II), Oxidation gave degradation product of FLUP at R_F values 0.40 ± 0.03 (FDP-I) (Fig. 4). No degradation products of FLUP and MELI were obtained after neutral, photo and heat stress conditions applied on both drugs individually or in combination. Physical appearance of FLUP was found to be change in photo stress condition.

toluene: chloroform: methanol: ammonia (4.5: 4.5: 1: 0.3 v/v) was therefore used as mobile phase and resulted in sharp, well defined, symmetrical peaks with no fronting when scanning was performed at 295 nm. There was no interference from common excipients present in the tablets. Linear ascending development to a distance of 80 mm was performed in a 10 cm × 10 cm CAMAG twin-trough chamber. Before the insertion of the plate, the chamber was saturated with mobile phase vapour for 10 min at room temperature and after the insertion of plate again saturated for 10 min. After development the plate was removed and dried with hot air drier. Densitometric scanning was performed at 295 nm with a CAMAG TLC Scanner III in reflectance–absorbance mode controlled by CATS 4 software (version 1.4.1; CAMAG) resident in the system. The slit dimensions were 4.00 mm × 0.45 mm and the scanning speed 20 mm s^{-1}. The radiation source was a deuterium lamp emitting continuous UV radiation between 190 and 360 nm. The amounts of the compounds chromatographed were determined from the intensity of diffusely reflected light.

Preparation of Sample Solution for Assay

Twenty tablets were weighed and finely powdered. An accurately weighed tablet powder equivalent to 0.5 mg of FLUP and 10 mg of MELI (154.3 mg) was transferred into a 10.0 ml volumetric flask. About 5.0 ml of methanol was added and mixture was sonicated for 10 min. The solution was cooled to room temperature and diluted up to the mark with methanol. The resultant solution was filtered through Whatman Grade I filter paper. Two milliliter of filtrate was transferred to a 10.0 ml volumetric flask and then volume was made up to the mark with methanol to obtain a concentration of 20 μg/ml and 400 μg/ml of FLUP and MELI respectively.

Six replicate of tablet powder equivalent to 0.5 mg of FLUP and 10 mg of MELI was transferred into six 10 ml volumetric flask and homogenous sample solutions were prepared in a similar manner.

Results and Discussion

HPTLC Method Development and Optimization

Normal phase HPTLC with toluene: chloroform: methanol: ammonia (4.5: 4.5: 1: 0.3 v/v) as mobile phase enabled satisfactory baseline resolution of the both API and all degradation products with reasonably acceptable R_F values for the purpose of quantification. R_F values were 0.45 ± 0.03 and 0.65 ± 0.03 for FLUP and MELI respectively, acidic hydrolysis gave degradation product of MELI at R_F value 0.53 ± 0.03 (MDP-I), alkaline hydrolysis gave degradation product of MELI at R_F values 0.58 ± 0.03 (MDP-II), Oxidation gave degradation product of FLUP at R_F values 0.40 ± 0.03

ISSN- 2394-5125 VOL 07, ISSUE 07, 2020

(FDP-I) (Fig. 4). No degradation products of FLUP and MELI were obtained after neutral, photo and heat stress conditions applied on both drugs individually or in combination. Physical appearance of FLUP was found to be change in photo stress condition.

3.2 Validation of the Method

As recommended in ICH guidelines [11,12] all validation was performed during development of the procedure. The proposed method was validated for linearity, precision, accuracy, specificity, limits of detection and quantification, ruggedness, and robustness. Linearity was established by least-squares linear regression analysis of the calibration data. Calibration plots were linear over the concentration range 50-250 ng by area for FLUP and 1000 - 5000 ng by area for MELI. Peak areas were plotted against the respective concentrations and linear regression analysis performed on the resulting curves. Equation for the calibration plots of FLUP was Y= 695.702 + 8.528 X, for peak area. Correlation coefficient was 0.98174 for peak area. Equation for the calibration plots of MELI was Y= 8172.224+ 2.202 X, for peak area. Correlation coefficient was 0.99107 for peak area. The limits of detection (LOD) and quantification (LOQ) were calculated from the standard deviation of the response and the slope of calibration plot. LOD and LOQ were established, in accordance with ICH definitions, by use of the equations LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the regression line and S is the slope of the calibration plot. The LOQ of FLUP for which precision and accuracy were satisfactory was 8.95 ng per band for peak area and LOD was 2.95 ng per band for peak area. The LOQ of MELI for which precision and accuracy were satisfactory was 16.62 ng per band for peak area and LOD was 5.48 ng per band for peak area.

Method, system and intermediate precision data are summarized in *Table I*. Method precision was investigated by injecting extracts from six tablet samples (n = 6) in triplicate. Intermediate precision (inter-day and intra-day) was investigated by injecting three samples (n = 3) in triplicate.

Accuracy data for the assay after analysis of the compound are summarized in Table 2. The accuracy of the method was determined on the basis of recovery studies performed by standard addition at different levels (80, 100, and 120%) of the label claim, in triplicate. A known amount of powder standard was added to samples of tablet powder, which was then mixed, extracted, and subsequently diluted to volume with AR-grade methanol, to yield the required concentration of drug. Specificity studies were conducted by attempting deliberate degradation of tablet samples by exposure in various stress conditions. The results showed in *Table III*. Ruggedness was assessed out for different elapsed times (intraday and inter-day). The results (*Table I*) showed the method is rugged under these conditions. Robustness was studied by varying the detection wavelength by ± 2.0 nm, mobile phase composition (± 0.2 ml of methanol) and saturation time by ± 5 min. The results are listed in *Table IV*.

ISSN- 2394-5125 VOL 07, ISSUE 07, 2020

Conclusion

The method enables simple, precise, and accurate analysis of flupentixol and melitracen and its degradation products in the bulk drug and pharmaceutical preparations. It was validated as per ICH guidelines. The method can therefore be used for routine quality-control analysis of flupentixol and melitracen in combined dosage forms.

Acknowledgments

The authors extend their sincere thanks Unichem Laboratories, Goa (India), for providing gift sample of pure flupentixol and melitracen respectively. We also extend our thanks to Head of Department, Department of Pharmaceutical Sciences; RTM Nagpur University for providing the necessary facilities

References:

1. M. Yunus, H. H. Siddiqui, P. Bagga, Md. A. Ali, K. Singh, Int. J. Pharm. Sci. Res. **2** (2011) 2152-2155.
2. B. Lakshminarayana, G. J. G. Babu, T. Menaka, Yogesh D. B., Int. J. ChemTech Res. 3 (2011) 580-583.
3. S. M. Hussain, M. Aqeel, S. A. Hussain, Int. J. PharmTech Res. 5 (2013) 1493-1497.
4. S. K. Acharjya, P. Panda, P. Mallick, K. R. Kumar, A. Narendra, Y. Sravani, Shilpa, M. M. Annapurna, J. Chem. Pharm. Res. **2** (2010) 158-171.
5. K. Patel, S. Mohan, American J. PharmTech Res. **3** (2013) 684-693.
6. U. K. Chhalotiya, K. K. Bhatt, D. A. Shah, G. R. Chauhan, S. L. Baldania, Chromatogr. Res. Int. 2011 (2011) 1-6.
7. S. Yi, L. Yan, Chinese Pharm. Affairs (2007) 894-896.
8. R. S. Limgavkar, P. D. Trivedi, A. J. Patel, J. Liq. Chromatogr. Related Technol. 12 (2012) 2753-2764.
9. U. K. Chhalotiya, K. K. Bhatt, D. A. Shah, C. D. Nagda, J. R. Patel, J. Liq. Chromatogr. Related Technol. **36** (2012) DOI: 10.1080/10826076.2012.685920.
10. M. C. Sharma, World Appl. Sci. J. **31** (2014) 165-170.
11. International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures, ICH-Q2A, Geneva, 1995.
12. International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: Methodology, ICH-Q2B, Geneva, 1996.

ISSN- 2394-5125 VOL 07, ISSUE 07, 2020

Table I

System, method, and intermediate precision data

Validation Parameters		FLUP			MELI		
		Mean	**SD [±]**	**RSD [%]**	**Mean**	**SD [±]**	**RSD [%]**
System Precision [a)]		4045.68	38.4732	0.9509	11909.09	27.5352	0.2312
Method Precision [a)]		100.38 %	0.1959	0.1951	100.03 %	0.3214	0.3213
Intermediate precision	**Intraday** [b)]	98.48 %	0.5273	0.5354	100.18 %	0.1137	0.1135
	Interday [b)]	98.36 %	0.5542	0.5634	100.14 %	0.1401	0.1399
	Different Analyst [b)]	100.27 %	0.2020	0.2015	100.04 %	0.1501	0.1500

a) Mean from six analyses (n =6)

b) Mean from 3 analyses (n =3)

n = Number of samples, SD = standard deviation; RSD = relative standard deviation

ISSN- 2394-5125 VOL 07, ISSUE 07, 2020

Table II

Accuracy data

Level	Wt. Of sample (mg)	FLUP					MELI				
		Amount of std. added (mg)	Area of sample	Area of standard	Amt. of std. recovered (mg)	% Recovery	Amount of std. added (mg)	Area of sample	Area of standard	Amt. of std. recovered (mg)	% Recovery
80%	77.0	0.20	1086.43	1250.31	0.196	98.00	4.0	7600.26	8554.98	3.98	99.50
80%	77.4	0.20	1092.91	1250.31	0.198	99.00	4.0	7621.93	8554.98	4.00	100.00
80%	77.2	0.20	1089.37	1250.31	0.197	98.50	4.1	7638.46	8554.98	4.02	98.04
100%	77.1	0.25	1209.36	1250.31	0.246	98.40	5.0	8447.31	8554.98	4.98	99.60
100%	77.2	0.25	1215.64	1250.31	0.248	99.20	4.9	8436.94	8554.98	4.97	101.42
100%	77.4	0.25	1221.94	1250.31	0.251	100.40	5.0	8462.49	8554.98	5.00	100.00
120%	77.2	0.30	1336.29	1250.31	0.298	99.33	6.0	9294.62	8554.98	5.98	99.66
120%	77.4	0.30	1343.69	1250.31	0.301	100.33	6.1	9329.61	8554.98	6.02	98.68
120%	77.1	0.30	1338.36	1250.31	0.298	99.33	6.0	9303.54	8554.98	5.99	99.83
					Average	99.16				**Average**	99.63
					SD [±]	0.8177				**SD [±]**	0.9331
					%RSD	0.8246				**%RSD**	0.9365

ISSN- 2394-5125 VOL 07, ISSUE 07, 2020

Table III
Specificity data

Formulation FLUTHIXOL	**Normal**	**Acid**	**Alkali**	**Neutral**	**Oxide**	**Heat**	**Photo**
FLUP [%]	101.29	101.59	100.38	101.23	95.35	100.54	100.23
MELI [%]	100.38	97.79	98.08	99.81	100.33	100.76	100.98

Table IV
Robustness

Condition		**FLUP**		**MELI**	
		By peak area*		**By peak area***	
		Amount estimated [%] ± SD	**RSD [%]**	**Amount estimated [%] ± SD**	**RSD [%]**
Change in wavelength (±2 nm)	**293 nm**	99.97 ± 0.2122	0.2122	99.79 ± 0.6792	0.6806
	297 nm	100.11 ± 0.1778	0.1776	99.97 ± 0.4186	0.4187
Change in mobile phase composition (±0.2 ml)	**Toluene: methanol: chloroform: ammonia 4.5: 0.8: 4.5: 0.3 (v/v)**	100.04 ± 0.5282	0.5280	100.00 ± 0.1126	0.1126
	Toluene: methanol: chloroform: ammonia 4.5: 1.2: 4.5: 0.3 (v/v)	100.10 ± 0.1069	0.1068	99.97 ± 0.1250	0.1250
Change in saturation time (±5min)	**15 min**	100.04 ± 0.3843	0.3841	100.09 ± 0.1514	0.1512
	25 min	100.46 ± 0.7369	0.7334	99.63 ± 0.7202	0.7228

* Each value is a mean of three observations.

ISSN- 2394-5125 VOL 07, ISSUE 07, 2020

Figure 1
Chemical structure of Flupentixol

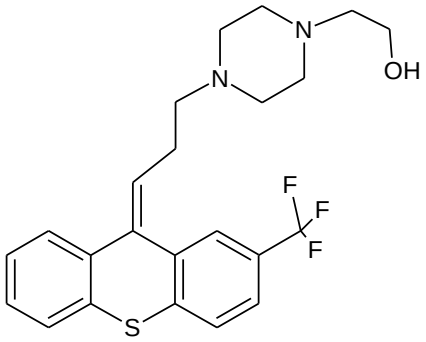


Figure 2
Chemical structure of Melitracen

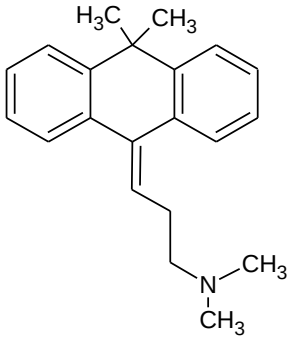
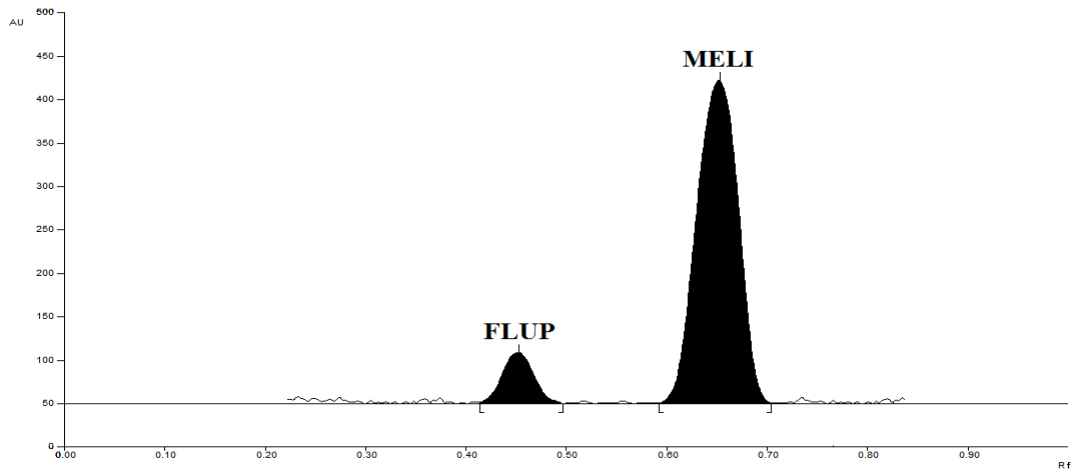


Fig. 3
Densitogram of Flupentixol and Melitracen combination



ISSN- 2394-5125 VOL 07, ISSUE 07, 2020

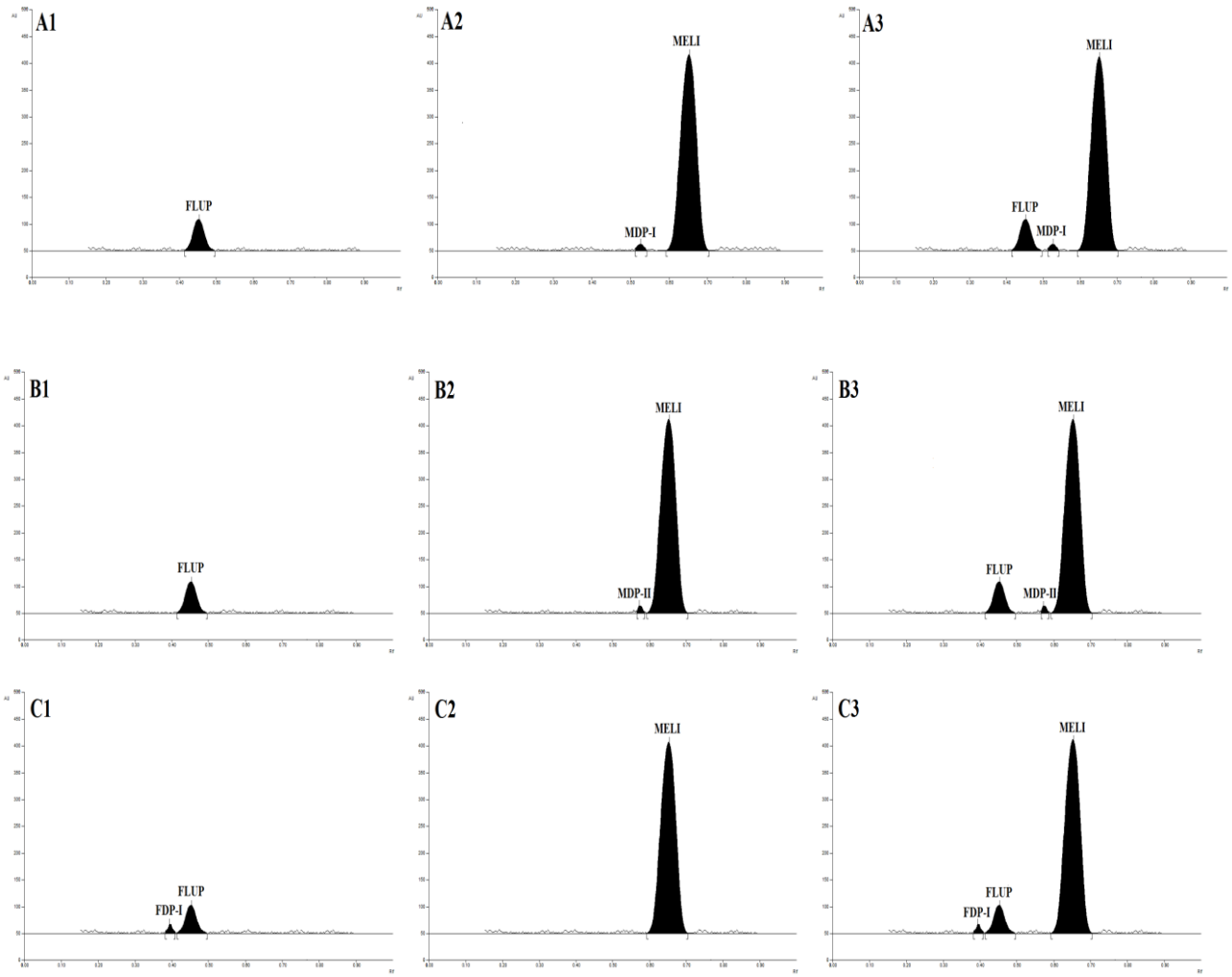


Fig. 4: Results from forced degradation of 1) Flupentixol, 2) Melitracen and 3) Tablet powder in A) 1 N HCL, 8 hrs. at 80^{o}C, B) 1 N NaOH, 8 hrs. at 80^{o}C , C) 3% H_2O_2, 1 hr. at room temperature

ISSN- 2394-5125 VOL 07, ISSUE 07, 2020

Captions

Table I

System, method, and intermediate precision data

Table II

Accuracy data

Table III

Specificity data

Table IV

Robustness

Fig. 1

Chemical Structure of Flupentixol

Fig. 2

Chemical Structure of Melitracen

Fig. 3

Densitogram of Flupentixol and Melitracen combination

Fig. 4

Results from forced degradation of 1) Flupentixol, 2) Melitracen and 3) Tablet powder in A) 1 N HCL, 8 hrs. at 80^{o}C, B) 1 N NaOH, 8 hrs. at 80^{o}C , C) 3% H_2O_2, 1 hr. at room temperature