A New Method Development and Validation of Rp-Hplc and Uv-Spectroscopy Method for the Simultaneous Estimation of Paracetamol and Mefenamic Acid in Bulk Drugs and Pharmaceutical Dosage Forms

V. Bhaskara raju[1], T. Yamini kumari[2],K. S. Sumanth[3,],P. Sri Tanvi[4]

[1]*Sri Vasavi Institute of Pharmaceutical Sciences, Pedatadepalli. Tadepalligudem, Andhra Pradesh, India-534101*

[3]*Corresponding author, Sri Vasavi Institute of Pharmaceutical Sciences, Pedatadepalli. Tadepalligudem, Andhra Pradesh-534101*

***Abstract*—The quantification of paracetamol and mefenamic acid in pharmaceutical dosage forms and bulk drugs was accomplished by the development and validation of a reverse-phase high-pressure liquid chromatographic technique (RP-HPLC). The two medications were separated chromatographically and analysed using a CAP CELL PAK C18 (250 X 4.6mm I.D., 5µm) column. Acetonitrile: methanol in a 90:10 ratio and acetonitrile:1% orthophosphoric acid in a 70:30 ratio make up the mobile phase, which has a gradient flow rate of 1.0 ml/min. At room temperature, absorbance at 230 nm was measured using a dual-wavelength UV detector. The retention periods of mefenamic acid and paracetamol were determined to be 4.538 and 2.695 minutes, respectively. The R2 correlation coefficients for mefenamic acid and paracetamol were 0.9963 and 0.9959, respectively.For both drugs, linearity was seen between 3.25 and 16.25 µg/ml and between 5 and 25 µg/ml. Mefenamic acid's and paracetamol's respective percentage RSDs were found to be. Assessments of accuracy conducted within and across days showed that the relative standard deviation (%RSD) of the proposed methods was below the maximum limit of 2.0. The proposed method was validated in accordance with ICH guidelines.The method for quantitatively analysing mefenamic acid and paracetamol in pharmaceutical formulations and bulk medications was found to be simple, economical, suitable, accurate, precise, and reliable.**

***Index Terms*—RP-HPLC, Paracetamol, Mefenamic acid, UV.**

I. INTRODUCTION

Paracetamol [PCT] is a white, crystalline, odorless substance that is chemically 4-hydroxy acetanilide. Bitter flavor. Saturated aqueous solution pH is around 6. (NTP, 1992). The impact of phenobarbital therapy on the biotransformation of parathion by intact mouse liver was studied, as well as the following impact of phenobarbital on parathion's acute toxicity. In male Hla (SW)BR Swiss Webster mice, daily intraperitoneal administration of 80 mg/kg phenobarbital for 4 days resulted in hepatic cytochrome p450 content, hepatic oxidative activation, and oxidative detoxification of parathion. This counteracted the acute toxicity of parathion without directly influencing tissue cholinesterase activities.Upon perfusing the livers of both control and phenobarbital-treated mice, paraoxon, p-nitrophenol, p-nitrophenyl sulphate, and p-nitrophenyl glucuronide were produced. The production of paraoxon was unaffected by phenobarbital, but it was increased in livers perfused with parathion.

Mefenamic acid [MEF] is a benzoic acid chemically known as 61-68-7 Ponstel 2-[(2,3-dimethylphenyl) amino]. By binding to the COX-1 and COX-2 prostaglandin synthetase receptors, mefenamic acid prevents prostaglandin synthetase from doing its job. These receptors momentarily lessen pain feelings because they play a part in prostanoid signaling in activity-dependent plasticity and/or as a significant modulator of inflammation.

The most used differentiation technique in HPLC today is reversed-phase chromatography because of its many uses. Over sixty-five percent (and probably as much as 90 percent) of all HPLC separations employ the reversed phase technique, according to predictions [12]. The development of a straightforward, accurate, and dependable reversed-phase HPLC technique for the simultaneous detection of paracetamol and mefenamic acid in pharmaceutical dosage forms and bulk drugs is the focus of the current study. High-performance thin-layer chromatography (HPTLC), UV spectrophotometry, and high-performance liquid chromatography (HPLC) are among the analytical methods that have been described in the literature for the identification of paracetamol and mefenamic acid in pharmaceuticals [13].

II. MATERIALS AND METHODS

INSTRUMENT:

UV Win software and a UV-Visible Spectrophotometer with 1 cm matched quartz cells are features of the UV-3092 LABINDIA double beam.

UFLC SHIMADZU Model: The LC-20AD is a manual injector-equipped UV-visible dual absorbance detector. Lab Solutions software was used to combine and monitor the output signal. For separation, a CAP CELL PAK C18 (250 X 4.6mm I.D., 5µm) column is employed.

Preparation of standard and sample solutions:

Standard stock solution of Paracetamol and Mefenamic acid:

After precisely weighing and transferring 10 mg of the working standards for paracetamol and mefenamic acid into two 10-ml volumetric flasks, they were dissolved in acetonitrile solution and reconstituted with the same solvent to yield a 1 mg/ml solution of each drug, respectively. Before analysis, the stock solutions were kept at -20 ± 20 °C in a refrigerator. Acetonitrile:0.1% orthophosphoric acid and acetonitrile:methanol (50:50) solutions were used to dilute the stock solutions to appropriate proportions in order to produce calibration curve (CC) standards and quality control (QC) samples.

Calibration curve standards and quality control samples:

Acetonitrile: Methanol and Acetonitrile:0.1% Orthophosphoric acid solution were used to adequately dilute the stock solutions to create working solutions for calibration and controls. This stock solution was diluted to yield concentration levels of 3.25, 6.5, 9.75, 13.0, and 16.25 µg/ml for paracetamol and 5, 10, 15, 20, and 25 µg/ml for mefenamic acid, respectively, which were used to create calibration standards for control samples. For paracetamol, quality control samples were generated in bulk at concentrations of 3.25µg/ml (50%-LQC), 9.75µg/ml (100%-MQC), and 16.25µg/ml (150%-HQC), and for mefenamic acid, at concentrations of 5µg/ml (50%-LQC), 15µg/ml (100%-MQC), and 25µg/ml (150%-HQC). Before being used, these samples were kept below -50 °C.

Preparation of mobile phase:

The mobile phase, which is composed of acetonitrile: methanol in a 90:10 ratio and acetonitrile:1% orthophosphoric acid in a 70:30 ratio, is sonicated for five minutes after being filtered through a 0.45µ µm membrane filter to exclude any contaminants that might affect the final chromatogram.

Selection of Detection Wavelength:

A solution of acetonitrile containing 10 µg/ml of paracetamol and 10 µg/ml of mefenamic acid was prepared to measure λmax. The spectra were recorded when it was scanned in the 200–400 nm range. Detection of UV at 205 and 213 nm. Detection of RP-HPLC at 230 nm. The isobestic point was used to choose a wavelength from the spectrum.

III. RESULTS AND DISCUSSION

A. Method development and optimisation:

The scanned absorption spectra of mefenamic acid and paracetamol were used to determine the detection wavelength. Separately, 10 mg of medicationwas dissolved in 10 ml of acetonitrile. Mefenamic acid and paracetamol's UV spectra were examined independently in the 200–400 nm wavelength range. The wavelength of 230 nm was chosen for examination following spectrum correlation.

Various columns (s, polar 18, Phenomenex, and capcell PAK c18) were used for the trials. Using a mobile phase made up of acetonitrile: methanol in a 90:10 ratio and acetonitrile:1% orthophosphoric acid in a 70:30 ratio, the medications were eluted at a gradient flow rate of 1.0 ml/min. It was discovered

that the retention periods for mefenamic acid and paracetamol were 4.538 and 2.695 minutes, respectively.

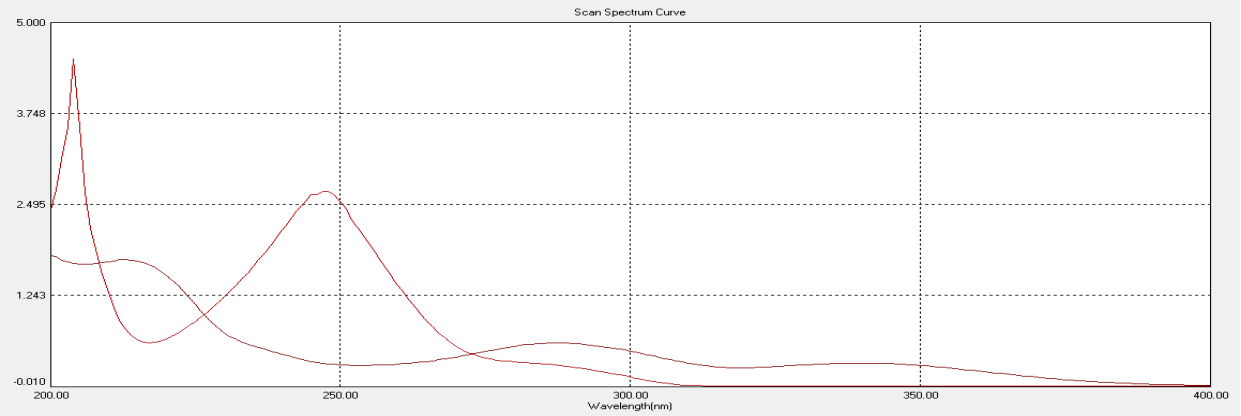

Fig 1: UV Overlay spectrum of Paracetamol and Mefenamic acid

B. VALIDATION

System suitability

According to ICH criteria, every system suitability metric was within the acceptable range. Six repetitions were given 10 µl of solution, which was then run into the HPLC system. The retention duration and standard deviations of each replication were analyzed, and the relative standard deviation was computed.

S.NO	Parameter	Paracetamol	Mefenamic acid
1.	Retention time	2.695	4.538
2.	Plate count	4790	9397
3.	Resolution factor	-	10.746
4.	Asymmetric factor	1.171	1.024
5.	%RSD	4.826	5.189

Acceptance Criteria: The plate count must exceed 2000, the resolution must exceed 2, and the tailing factor must be less than two, per ICH norms. Every appropriate parameter of the system was passed and was inside the bounds.

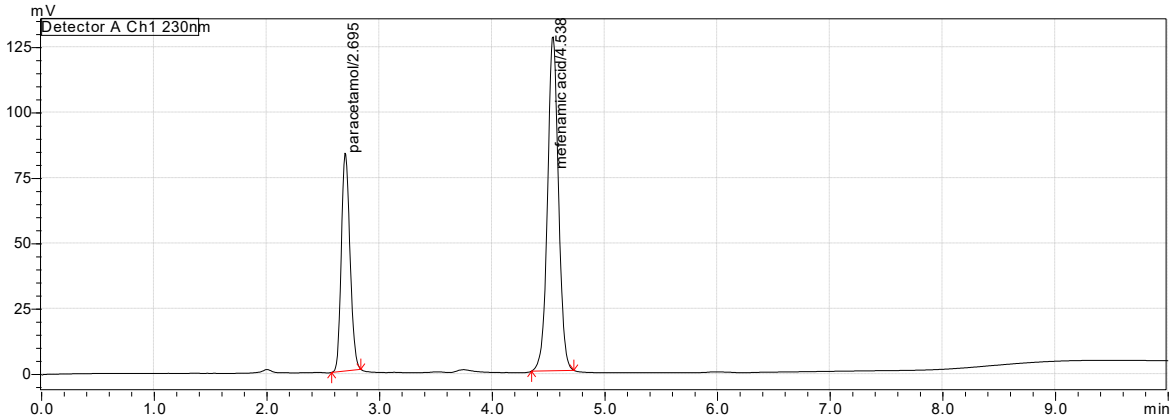

Figure 2: standard chromatogram

SENSITIVITY:

LOD and LOQ are used to express the method's sensitivity. Using the signal-to-noise ratio approach, it was computed. Concentrations with S/N ratios of 3:1 are LOD, and those with S/N ratios of 10:1 are LOQ.

DRUG	LOD	LOQ
Paracetamol	0.03µg/ml	1 µg/ml
Mefenamic acid	0.5µg/ml	0.1 µg/ml

LINEARITY:

For paracetamol, linearity was established over the range of 3.25–16.25 µg/ml, and for mefenamic acid, 5–25 µg/ml. Linearity graphs with time on the x-axis and concentration on the y-axis were displayed as Figures 3a and 3b, respectively, using the weighted least square regression analysis. The results were displayed in Table 2.

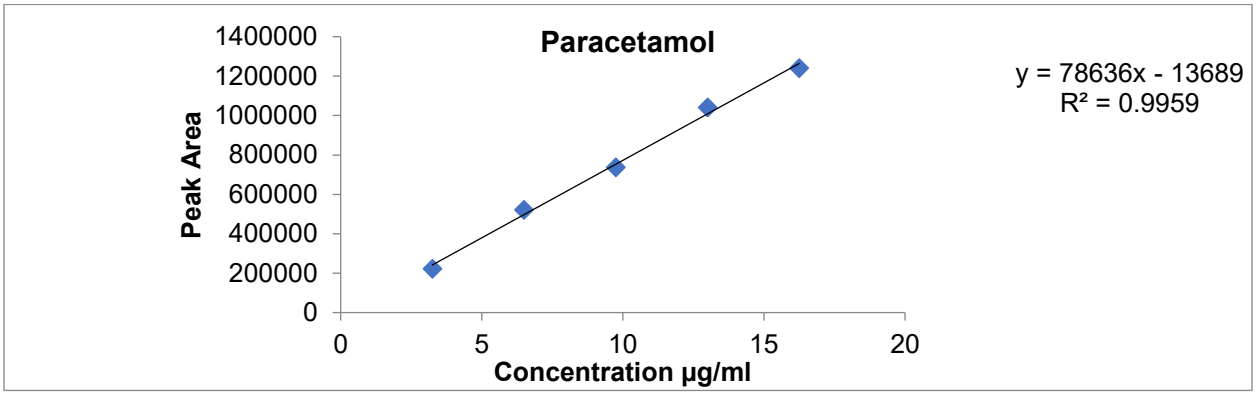

Fig 3a:Linearity plot of Paracetamol

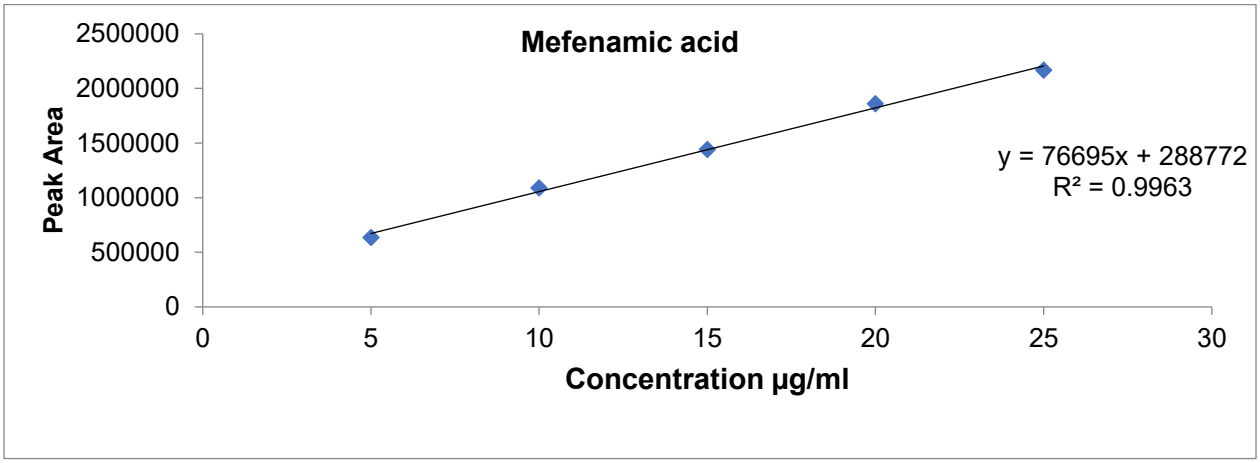

Fig 3b: Linearity plot of Mefenamic acid

Table 2: Linearity of Paracetamol and Mefenamic acid

S.NO	Paracetamol		Mefenamic acid	
	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
1	3.25	223288	5	636107
2	6.5	521613	10	1090301
3	9.75	737939	15	1440332
4	13.0	1040557	20	1861179
5	16.25	1241643	25	2168032

Table 2: graph properties

Regression equation	y = 78636x - 13689	y = 76695x + 288772
Slope	78636	76695
Intercept	13689	288772
R2	0.9959	0.9963

PRECISION:

One volumetric flask of working standard solution was used to make three injections; the regions that resulted are mentioned above. The average area, standard deviation, and % RSD were calculated for two drugs. The percentage RSDs for mefenamic acid and paracetamol were 0.65% and 1.81%, respectively. This procedure passed the system precision because the precision limit was less than "2". The system, technique, and intermediate precisions of mefenamic acid and paracetamol were found to be within acceptable limits. The results were shown in Tables 3a, 3b, 3c, 3d, 3e, and 3f.

Table 3a: Intra-runprecision of Paracetamol

PARACETAMOL			
SAMPLE NO.	LQC	MQC	HQC
1	491272	462240	517792
2	489282	473252	528645
3	513956	485493	548931
AVG	498170	473661.7	531789.3
SD	11191.91	9497.416	12905.41
RSD	2.246605	2.005106	2.42679

Table 3b: Intra-run precision of Mefenamic acid

MEFENAMIC ACID			
SAMPLE NO.	LQC	MQC	HQC
1	500538	526713	641345
2	519468	534163	652456
3	528395	563189	664568
AVG	516133.7	541355	652789.7
SD	11614.4	15735.7	9483.685
RSD	2.25027	2.906724	1.452793

Table 3c: Intra-Day Precision of Paracetamol

PARACETAMOL			
PRECESION	LQC	MQC	HQC
1	301588	331327	516976
2	301107	337640	521680
3	291272	316986	532346
AVG	297989	328651	523667.3
SD	4753.694	8641.669	6430.207
RSD	1.595258	2.629436	1.227918

Table 3d: Intra-Day Precision of Mefenamic acid

MEFENAMIC ACID			
SAMPLE NO	LQC	MQC	HQC
1	493060	512390	513329
2	510277	548441	537441
3	524079	550075	550063
AVG	509138.7	536968.7	533611
SD	12689.01	17392.54	15239.17
RSD	2.49225	3.239023	2.855857

Table 3e: Inter-Day Precision of Paracetamol

PARACETAMOL			
	LQC	MQC	HQC
1	1118341	113205	1244735
2	1114375	112240	1275247
3	1151990	115366	1302229
AVG	1128235	113603.7	1274070
SD	20667.5	1600.678	28765.06
RSD	1.831843	1.409002	2.257729

Table 3f: Inter-Day Precision of Mefenamic acid

MEFENAMIC ACID			
	LQC	MQC	HQC
1	313301	312001	222065
2	320964	321249	228790
3	321509	300431	221600
AVG	318591.3	311227	224151.7
SD	4589.66	10430.56	4023.637
RSD	1.44061	3.351432	1.795051

ACCURACY:

A crucial validation criterion in the development of HPLC (High-Performance Liquid Chromatography) methods is accuracy, which establishes how closely the measured values match the actual or recognized reference value. It guarantees that the procedure can yield findings that accurately represent the analyte's true content in the sample. The degree of agreement between the value discovered and the actual value or a standard (such as a reference material or known concentration) is referred to as accuracy. To do this, a known quantity of standard (spiked) analyte is added to the sample matrix. Utilizing the following formula, determine the percentage recovery after preparing the samples at the LQC, MQC, and HQC concentration levels.

Recovery (%) = (Added Concentration / Observed Concentration) ×100

Table 4a: Accuracy studies of Paracetamol

Concentration level	Concentration of the sample	Amount of spiked standard	Amount recovered	% Recovery
LQC (µg/ml)	3.25	5	3.16	97.09
MQC (µg/ml)	6.5	5	6.14	98.19
HQC (µg/ml)	9.75	5	9.54	97.82

Table 4b: Accuracy studies of Mefenamic acid

Concentration level	Concentration of the sample	Amount of spiked standard	Amount recovered	% Recovery
LQC (µg/ml)	5	5	9.86	98.61
MQC (µg/ml)	15	5	19.60	98.02
HQC (µg/ml)	25	5	28.98	96.56

ROBUSTNESS:

To evaluate the resilience of the approach, optimal chromatographic conditions were purposefully altered; the mobile phase B ratio (ACE: orthophosphoric acid) and flow rate (1 ml/min) were adjusted by ± 2%. Additionally, robustness was examined by varying the flow rate with the real ratio to 1.1 ml/min, 1.0 ml/min, and 1.3 ml/min, respectively. Two robustness assessments were conducted using doses of 9.75 µg/ml of paracetamol and 15 µg/ml of mefenamic acid.

Table 5a: Robustness table of Paracetamol

Parameter	Condition	Retention time(min)	Peak area	Tailing	Plate count
Flow rate Change (ml/min)	Less flow than actual (1.1ml)	2.687	732489	1.09	3690
	More flow than actual (1.3ml)	2.687	697816	1.07	3520
Organic Phase Change	Less Org (A – 88:12, B –68:32)	2.708	720477	1.09	3690
	More Org (A – 92:8, B – 72:28)	2.687	697816	1.07	3520

Table 5b: Robustness table of Mefenamic acid

Parameter	Condition	Retention time(min)	Peak area	Tailing	Plate count
Flow rate Change (ml/min)	Less flow than actual (1.1ml)	4.689	1562314	1.01	8286
	More flow than actual (1.3ml)	4.443	1540549	1.01	8176
Organic Phase change	Less Org (A – 88:12, B-68:32)	4.582	1592970	1.01	8286
	More Org (A – 92:8, B –72:28)	4.415	1649484	1.01	8176

ASSAY:

Fill a 100 ml volumetric flask with five tablets of paracetamol and mefenamic acid, each of which contains 325 mg of paracetamol and 500 mg of mefenamic acid as stated on the package. 50 ml of mobile phase in equal amounts was sonicated for 15 minutes. Next, use the same mobile phase to make up to 100ml. To create 100, transfer 1 milliliter of the stock solution mentioned above to a 10-milliliter volumetric flask. Then, repeat the process to create 10 µg/ml. Into the HPLC, inject.

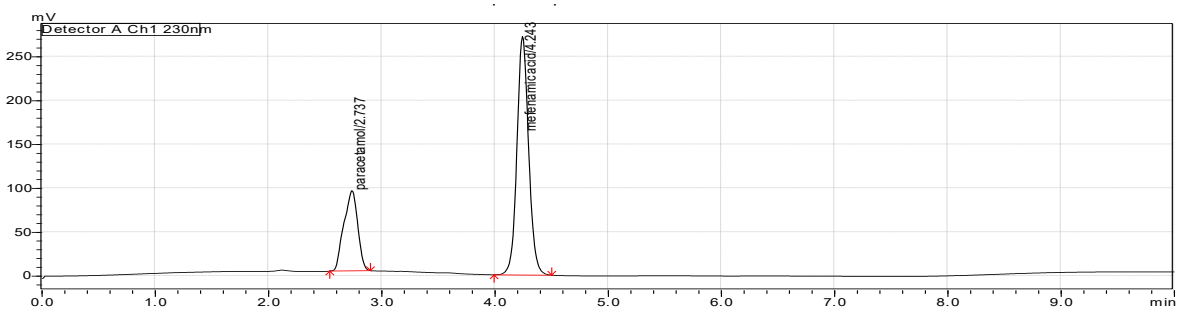

Fig 4: sample chromatogram

IV. CONCLUSION

The HPLC method is simple to use, fast, accurate, precise, reliable, and efficient. It was developed for the assessment of various drugs. The mobile phase and solvents are affordable, reliable, sensitive, and simple to prepare. Because the system validation parameters—linearity, precision, robustness, and assay of the HPLC method used for estimation of selected drugs in pure form—have also demonstrated adequate, precise, and reproducible results, it is determined that the brief and simple suggested methods are the most beneficial for analysis purposes.

Thus, these are relevant to the routine examination of paracetamol and mefenamic acid.

REFERENCES

[1] Satyanarayana;Dondeti; Kannan; Kamarajan; Manavalan; Rajappan, Journal of the Serbian Chemical Society (2006), 71(11), 1207-1218.

[2] Dinc; Erdal; Yucesoy; Cem; Onur, Feyyaz, J. Pharm. Biomed. Anal (2002), 28(6), 1091- 1100.

[3] Thai, Duy Thin; Nguyen; Tuong Vy; Tran, Viet Hung. Tap Chi Duoc Hoc (2006), 46(2), 27-31.

[4] [4] Gangwal; Shrenik; A. K. Sharma, Indian J. Pharma Sci. (1996), 58(5), 216-218.

[5] Das, Sukomal; Sharma; C Suresh; Talwar; K Santosh; P. D Sethi, Analyst (Cambridge, United Kingdom) (1989), 114(1), 101-3.

[6] Madrakian; Tayyebeh; Afkhami; Abbas; Mohammadnejad; Masoumeh; Analytica Chimica Acta (2009), 645(1-2), 25-29.

[7] Hung, Chin-Yin; Hwang, Ching-Chiang, J. Chroma. Sci. (2008), 46(9), 813-818.

[8] Jaiswal; Yogini; Talele; Gokul; Surana; Sanjay, Journal of Liquid Chromatography & Related Technologies (2007), 30(8), 1115-1124.

[9] E. Mikami; T Goto; T. Ohno; H Matsumoto; K Inagaki; H. Ishihara; M. Nishida, J. Chromatogram B: Biomed Sci and Applications (2000), 744(1), 81-89.

[10] Rau; L. Harish; A. R Aroor; Rao; P Gundu, Indian Drugs (1991), 28(12), 563-5.

[11] MadhukarA; V. Sudhirkumar;P. Anand; C.H. Samrat, J.Chem. Pharma. Res. (2011), 3(3), 464-469

[12] D.K. Mandloi, P.K. Tyagi, V.K. Rai, S. Dey, R.K. Ashada, and P. Mohanraj J. Chem. Pharma. Res. (2009),1(1), 286-296

[13] S. R. Pattan, S. G. Jamdar, R. K. Godge, N. S. Dighe, A.V. Daithankar, S. A. Nirmal, and M.G. Pai, J. Chem. Pharma. Res. (2009),1(1), 329-335

[14] Maliye; N Amit; Walode; G Sanjay; Kasture; V Avinash; Wadodkar; G Sudhir, Asian J Chemistry (2005), 18(1), 667-672.

[15] A.P. Argekar; J G Sawant, Journal of Planar Chromatography— Modern TLC (1999), 12(5), 361-364. [16] ICH Q2A, Text on validation of analytical procedures, International Conference on Harmonisation tripartite guidelines, adapted 27 Oct 1994B