JCBPS; Section A; August 2017 – October, 2017, Vol. 7, No. 4; 919-929. E- ISSN: 2249 –1929

[DOI: 10.24214/jcbps.A.7.4.91929.]

Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section A: Chemical Sciences

CODEN (USA): JCBPAT Research Article

Method Development and Validation of Combination of Methocarbamol and Ibuprofen Using RP-HPLC

N.Ramalakshmi*[1], Savita[1], C.N.Nalini[1] and S.Arunkumar[2].

[1]C.L.Baid Metha college of Pharmacy. Chennai

[2]College of Pharmacy.Gulf medical University. Ajman, UAE.

Dr.N.Ramalakshmi Professor and Head Pharmaceutical Chemistry C.L.Baid Metha college of Pharmacy. Chennai-600097

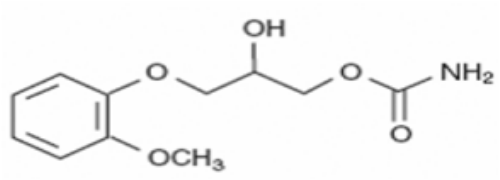
Received: 23 August 2017; **Revised:** 04 September 2017; **Accepted:** 10 September 2017

Abstract: A new simple, rapid and sensitive isocratic RP-HPLC method has been developed for the determination of methocarbamol and ibuprofen. The method employs shimadzu HPLC system on ODS HG5 column. Best chromatographic separation was achieved by using orthophosphoric acid : methanol : Acetonitrile in the ratio of 15:45:40 as mobile phase with a flow rate of 1ml/min and isocratic elution with a total run time of 30 minutes. Detection of the compound was carried out at 230nm. The retention times of methocarbamol and ibuprofen were found to be 2.8 and 3.6 respectively. The linearity studies range from at the concentration range of 80 -120 µg/ml. This method was validated for accuracy, precision, linearity, ruggedness and robustness as per ICH guidelines. The present newly developed method was found to be accurate, precise and can be useful for routine Quality control analysis.

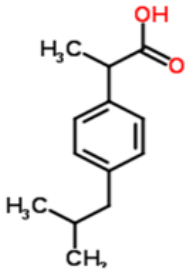
Keywords: methocarbamol, ibuprofen, HPLC estimation, analytical method development, validation.

INTRODUCTION

Methocarbamol is a potent muscle relaxant, act by blocking nerve impulses that are sent to brain. Methocarbamol is used together with rest and physical therapy to rest skeletal muscle such as pain or injury. Comes in the dose of 500mg. its plasma clearance ranges between 0.2-0.8 L/h/kg, so half life ranges between 1 and 2 hours, and the plasma protein binding ranges between 46% and 50% its metabolites are eliminated in urine and small unchanged methocarbamol also are excreted in the urine. The most side effects are anaphylactic reaction, angioneurotic edema, fever, headache, bradycardia, flushing, hypotension, syncope, dyspepsia, jaundice, vomiting , leucopenia, hypersensitivity reactions, amnesia, confusions, dizziness, drowsiness , insomnia, sedation, vertigo, rashes and urticarial. Toxicity can be treated with adequate airway, monitoring urinary output and vital signs, and administration of fluids if necessary.



structure of methocarbamol



structure of ibuprofen

Ibubrofen exhibits anti-inflammatory, analgesic and antipyretic activities. Its analgesic effect is independent of anti-inflammatory activity and has both central and peripheral effects. Literature surveys reveal that only few methods have been described for analysis of methocarbamol and ibuprofen[1-8].

EXPERIMENTAL

Instrumentation: Different kinds of equipmentsviz analytical weighing balance (shimadzu AUX 200), High performance liquid chromatography (Agilent-1200 series) equipped with Auto Sampler and UV detector. Column Symmetry C18 (4.6 x 250mm, 5□m, Make: X-bridge), pH meter, Vacuum filter pump (model XI 5522050 of Millipore), Millipore filtration kit, mobile phase reservoir, Water bath, Sample filtration assembly and glassware's were used throughout the experiment.

Chemicals and solvents: Acetonitrile, Methanol were used as HPLC grade, Potassium di hydrogen phosphate was AR grade, Water was Milli-Q-grade, Sodium hydroxide-GR grade, Ortho phosphoric acid -LR grade were used and Purchased from Merck Specialties Private Limited, Mumbai, India.

Preparation of buffer: Weighed 10.89grams of KH_2PO_4 into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. Adjust the pH to 4.5 with orthophosphoric acid. Filter through 0.45µm nylon membrane filter and degas.

Mobile phase: pH 4.5 buffer potassium dihydrogen phosphate, Methanol and Acetonitrile (15:45:40)

Preparation of Methocarbamol Standard stock solution-A: Accurately weigh and transfer 100 mg of methocarbamol working standard into a 10ml clean dry volumetric flask add about 7ml of diluents and sonicated to dissolve it completely and made volume up to the mark with the diluents.

Preparation of Ibuprofen Standard stock solution-B: Accurately weigh and transfer 10 mg of Ibuprofen working standard into a 10ml clean dry volumetric flask add about 7ml of diluents and sonicated to dissolve it completely and made volume up to the mark with the diluents

.**Preparation of Mixed Standard stock solution-C:**1 ml of standard solution-A and 4 ml of standard solution-B was pipetted into the 10 ml volumetric flask and mixed well. It gave the concentration Methocarbamol -1000ppm and Ibuprofen 400 ppm

Preparation of Formulation Solution

Stock-1: 20 Tablets were weighed and powered and calculated the average weight. 1.632g of tablet powder was weighed and transferred into the 100 ml volumetric flask and added the 70ml of mobile phase, mixed well made up to 100ml with the mobile phase.

Stock-2: 2ml of above Stock-1 solution was pipette into the 100 ml volumetric flask and added the 70ml of mobile phase, mixed well made up to 100 ml with the mobile phase which is equivalent to 100 ppm of Methocarbamol and 40 ppm of Ibuprofen.

Preparation Chromatographic parameters:

Equipment	: High performance liquid chromatography equipped with auto sampler and UV detector
Column	:C18 (4.8X250mm,5µm,make:X-bridge)
Flow rate	:0.8mL/min
Wavelength	:230 nm
Injection volume	: 10 min

METHOD DEVELOPMENT

To develop a simple and robust method for the simultaneous determination of methocarbamol and ibuprofen in combined tablet dosage form using HPLC. The spectra of diluted solution of the methocarbamol and ibuprofen were recorded separately on UV spectrometer. The peak of maximum absorbance wavelength was observed and was found tobe 230nm.

Initial development trials have performed with octyl and octadecyl columns with different types, configurations and from different manufacturers. Finally good separation of both the drugs wereachieved in X-bridge C18 column (250X4.6mm,5µm)in this column both the drugs were resolved and symmetric peaks with high theoretical plates and low tailing factor was observed.

To effect ideal separation of the drug under isocratic conditions, mixtures of solvents like buffer, methanol and Acetonitrile with or without different combinations were tested as mobile phases on X-bridge BDS C18 stationary phase. A mixture of methanol, Acetonitrile and buffer in the ratio of

15:45:40(v/v) was proved to be the most suitable of all the combinations since chromatographic peaks obtained were better defined and resolved and almost free tailing.

Flow rates of the mobile phase were changed from 0.5-1.2 mL/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 0.8 ml/min flow rate was ideal for the successful elution of the analyte.

No interference in blank and placebo solutions for both drug peaks in the trail injections with a run time of 10 min. The above optimized chromatographic conditions were followed for the simultaneous determination of methocarbamol and ibuprofen formulations.

Method validation: A new optimized method suitable for the simultaneous routine analysis of methocarbamol and ibuprofen in pharmaceutical formulation samples was successfully developed. The developed method was validated as per ICH guidelines.

Calibration curve was stabled by preparing six different concentrations of methocarbamol and ibuprofen based on the label claim of the formulation dosage form. Which is equivalent to 100 ppm of Methocarbamol and 40 ppm of Ibuprofen solutions were mixed, from this 10µl of the sample was injected In to HPLCsystem. Peak area responses of the corresponding standards were used in constructing the calibration curve.

Accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of preanalysed tablet solution. Percent recovery was calculated comparing the area before and after the addition the standard drug. The standard addition method was performed at 50%, 100% and 150% levels of standard concentration for both Methocarbamol and Ibuprofen the solutions were analyzed in triplicate at each level as per the proposed method.

Precision is the measure of how close the data values to each other for a number of measurements under the same analytical conditions. Precision of the method was determined by performing interday variation and repeatability studies. Standard concentration of Methocarbamol and Ibuprofen was prepared in six times and %RSD was calculated in both intraday and interday precision.

The evolution of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reality of an analysis with respect to deliberate variations in method parameters. Robustness test as carried out by small variation in the chromatographic conditions and change in the results was carried out by small variation in the chromatographic conditions and change in the results as calculated. Here robustness was performed by change in mobile phase ratio mobile phase pH and wavelength of the detector. Standard solution was analysed under these changed experimental conditions. % change in the results was calculated

Inter day variations were performed by using six replicate injections of standard solution which were relative standard deviation (% RSD) was calculated. Determination of the signal-to-noise ratio is performed by measured signals from samples with concentration at which the analyte can be reliably detected. A signal-to-noise ratio 2:1 is generally considered acceptable for estimating the detection limit. The quantification limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

Analysis of tablet formulation: The prepared formulation solution was injected into HPLC system. Peak areas of the corresponding peak area compared with the standard results and formulation assay was calculated for the proposed method.

RESULTS AND DISCUSSION

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drug products. The objective of this study was to develop a rapid and sensitive HPLC method for estimation of methocarbamol and ibuprofen in tablet formulation using the most commonly employed RP-C18 column withy UV detection. The mobile phase was optimized with (15:45:40)from the overlain spectrum of methocarbamol and ibuprofen the wavelength was selected at 230 is isoabsorptive point for both drugs. Good resolution was carried out at 230nm and both drugs showed good absorbance at this wavelength with minimum interference of the other drug. Optimized chromatographic conditions were shown in **Table-1**. Standard and blank and formulations chromatogram were shown In **Figure-1 and 2**. All parameters of these proposed method was validated as per the ICH guidelines.

Table-1: Optimized chromatographic conditions for Methocarbamol and Ibuprofen

S.No	Parameter	Results
1	MP	Methanol: Acetonitrile: buffer in 15:45:40 (v/v)
2	Wavelength	230 nm
3	Stationary phase	X-bridge BDS C-18 column
4	pH of mobile phase	4.5
5	Flow rate	0.8ml/min
6	Run time	10min
7	Pump mode	Isocratic
8	Pump pressure	15.5±5MPa
9	API concentrations	Methocarbamol -100 ppm and Ibuprofen -40 ppm
10	RT	Methocarbamol -2.83and Ibuprofen -3.64
11	Resolution	
12	Area	Methocarbamol -244809and Ibuprofen -312542
13	Theoretical plate	
14	Tailing factor	

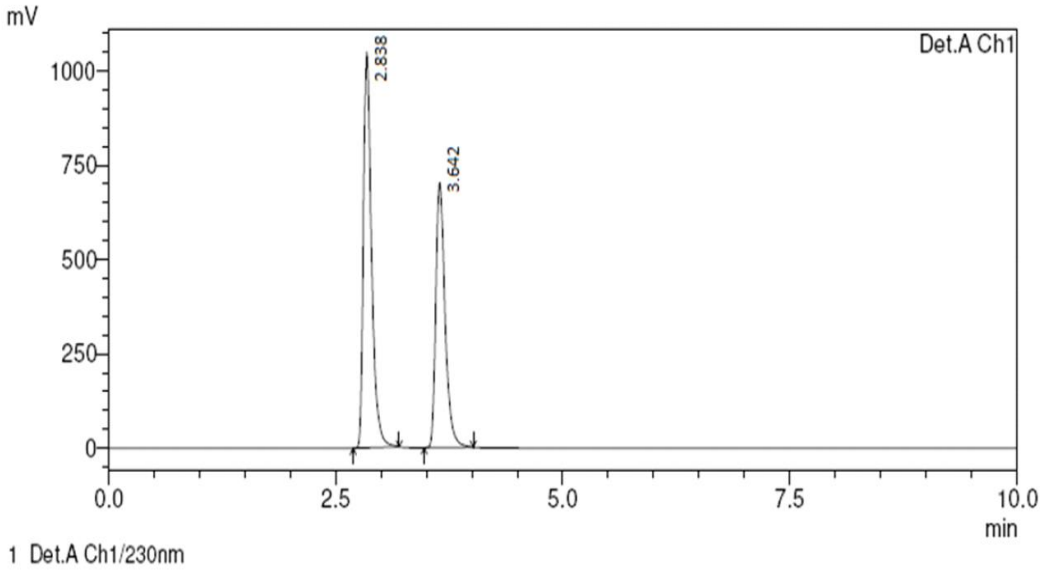


Fig.1: Standard chromatogram of methocarbamol and ibuprofen

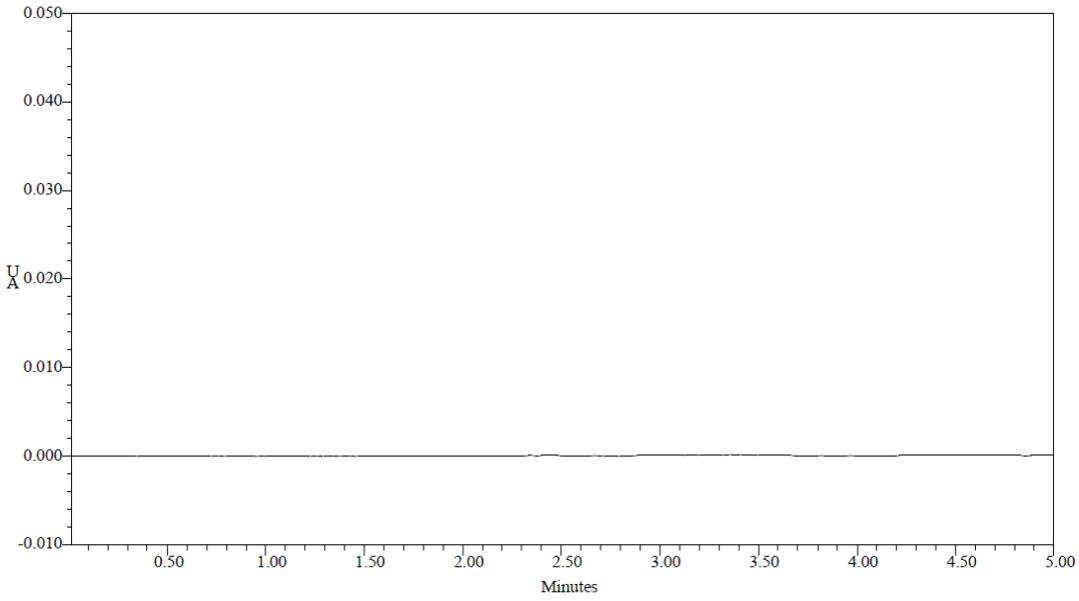


Fig.-2: chromatogram of blank

Blank and placebo solutions show no peak at the retention time of methocarbamol and ibuprofen, hence proving the specificity of the method. A good linear relation was observed with in concentration range of 25-150µg/ml for methocarbamol with regression equation y=23603.254X43561.7 [r^2 0.999] and 10-60µg/ml for ibuprofen with regression equation y=8894.685x-42626.3[r^2 0.9991]. linearity results were shown in **Table 2** and graphs were shown in **Fiqure-4**

Recovery was carried out by standard addition method of 50%,100%, addition to standard , pre analysed sample of 100µg/ml for methocarbamol in triplicates. % recovery for each case was calculated and was found to be within the acceptance criteria of 98-102 % for both drugs. This showed that the recoveries of

Table-2: Linearity results of methocarbamol and ibuprofen

S.No	Methocarbamol		Ibuprofen	
	Concentration [µg/ml]	Peak Area	Concentration [µg/ml]	Peak Area
1	25	552362	10	39913
2	50	1102365	20	141121
3	100	2381321	40	319012
4	125	285463	50	399030
5	150	3482145	60	488836

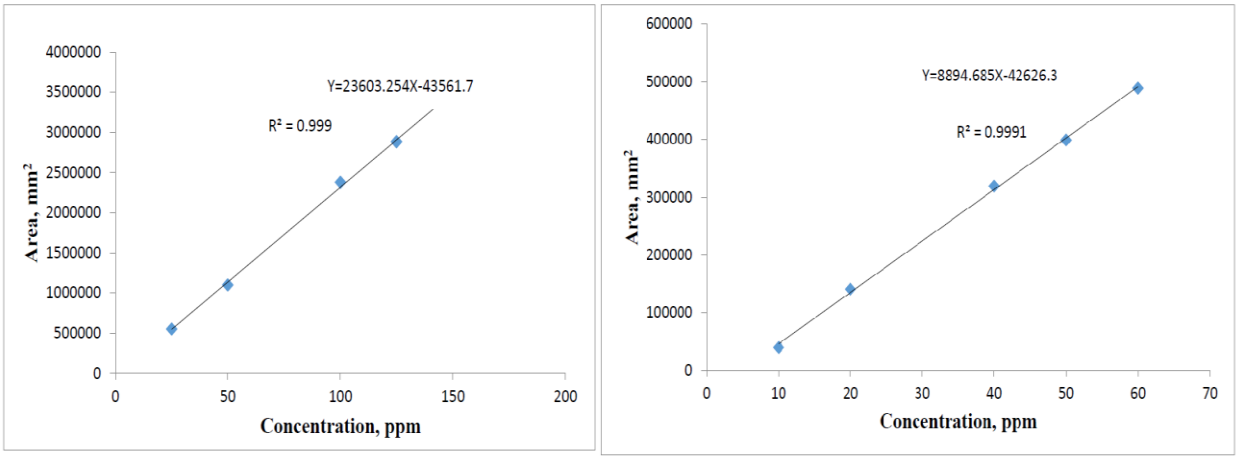


Fig.4: Linearity of methocarbamol and ibuprofen

Methocarbamol and ibuprofen by proposed methods are satisfactory. Recovery results were shown in **Table 3&4**.The precision was measured in terms of repeatability, which was consequent three days for inter day precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample in the day (intraday) and next consequent three days for inter day

Table-3: Recovery results of methocarbamol

S.No	% Level	Area	Amount Spiked +Sample	% Recovery	Mean Recovery	% SD
1	50	1216587	1224034	99.39	100.12	0.83
2		1236534	1224034	101.02		
3		1223564	1224034	99.96		
4	100	2442351	2448069	99.77	99.81	0.16
5		2447702	2448069	99.99		
6		2440321	2448069	99.68		
7	150	3600012	3604501	99.88	100.21	0.38
8		3608965	3604501	100.12		
9		3627452	3604501	100.64		

Table-4: Recovery results of ibuprofen

S.No	% Level	Area	Amount Spiked + Sample	% Recovery	Mean Recovery	%SD
1	50	154369	156271	98.78	98.97	0.37
2		155321	156271	99.39		
3		154301	156271	98.74		
4	100	312654	312542	100.04	101.49	1.25
5		318974	312542	102.06		
6		320014	312542	102.39		
7	150	465238	468813	99.24	99.42	0.73
8		463257	468813	98.81		
9		469823	468813	100.22		

Recovery was carried out by standard addition method of 50%,100%, addition to standard , pre analysed sample of 100µg/ml for methocarbamol in triplicates. % recovery for each case was calculated and was found to be within the acceptance criteria of 98-102 % for both drugs. This showed that the recoveries of methocarbamol and ibuprofen by proposed methods are satisfactory. Recovery results were shown in **Table 3&4**.The precision was measured in terms of repeatability, which was consequent three days for inter day precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample in the day (intraday) and next consequent three days for inter day precision.

For each cases% RSD as calculated and as found to be 1.6 and 1.06 for ibuprofen in interday precision 0.29 and 0.39 for methocarbamol and ibuprofeninter day and interday precision. Thiswas lying within the acceptable range of ±2. This showed that the precision of the methods are satisfactory.

Ruggedness performed by using six replicate injections of standard solution of concentrations which were prepared and analysed by different analyst on three days over a period of one. The percent relative %RSD was calculated and and it was found to be 0.87 and 0.43 for Methocarbamol and ibuprofen respectively, which are well within the acceptable criteria of not more than 2.0. It was concluded that the analytical technique showed good repeatability. Results were shown in **Table-5**.The limit of detection values was found to be 5.44µg/ml methocarbamol and 2.07µg/ml for ibuprofen and limit of quantification values was found to 16.48µg/ml methocarbamol and 6.26µg/ml for ibuprofen. This indicates that the proposed method is very sensitive.

Table-5: Ruggedness of methocarbamol and ibuprofen

S.No	Methocarbamol			Ibuprofen		
	Intraday	Interday	Ruggedness	Intraday	Interday	Ruggedness
Injection 1	2371329	240142	2371321	319215	317111	317012
Injection 2	2400011	239875	2394321	317625	320101	317112
Injection 3	2396598	238954	2394321	318652	318624	317019
Injection 4	2309875	234158	2400011	319526	319852	320101
Injection 5	2395867	239845	2350869	320022	319862	317183
RSD	1.60	1.06	0.87	0.29	0.39	0.43

Small deliberate variation in the chromatographic condition does not show influence on the results indicates that the proposed method is robust. Results of the robustness were shown in **Table-6**.The validated method was applied for the assay of commercial tablets of methocarbamol and ibuprofen. area of the detector response was used to calculate % assay. The results presented good agreement with the labeled content. Results were shown in **Table-7** and formulation chromatogram was shown in **figure- 3**.

Table-6: Robustness results of methocarbamol and ibuprofen

S.NO	Condition	Change	Methocarbamol		Ibuprofen	
			RT	AREA	RT	AREA
1	Standard	NA	2.840	2448069	3.652	312542
2	Flow rate 1	0.9 ml/min	3.339	3400011	4.138	420101
3	Flow rate 2	0.7 ml/min	3.290	3400020	4.08	420113
4	RSD	-	1.04	0.00019	0.998	0.002
5	Temperature 1	40ºC	2.840	2394321	3.642	317019
6	Temperature 2	30ºC	2.842	2371321	3.643	317012
7	RSD	-	0.049	0.6825	0.019	0.00156

Table-7: Formulation results of methocarbamol and ibuprofen.

S.NO	DRUG	DOSAGE(mg)	Amount present(mg)	% ASSAY
1	Methocarbamol	750	746.92	99.59
2	Ibuprofen	400	399.16	99.79

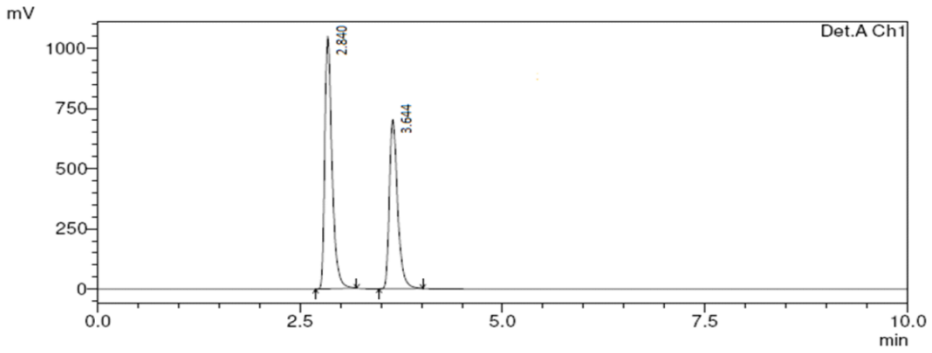


Fig.-3: Formulation chromatogram of methocarbamol and ibuprofen

CONCLUSION

A simple isocratic RP-HPLC method has been developed for the simultaneous determination ofmethocarbamol and ibuprofen using a UV detector. The developed method contains buffersin the mobile phase with simple UV detection with less mobile phase flow rate. Hence the method is simple, sensitive, accurate, rugged, robust, rapid and precise. The absence of additional peaks in the chromatogram indicated that there is no interference of the common excipients used in the tablets. The method has a relatively short run time that allows quantifying a large number of samples in routine and quality control analysis of fixed dose combination tablets. Hence, the above said method can be successfully applied for the simultaneous estimation of methocarbamol and ibuprofen in tablet dosage forms.

.REFERENCES

1. RB.PrasannaInt. JornalChemTech Research. 2009, 1(3), 499-501.
2. PK.Nallasivan, KC.Saranya, R.SivaKumar, S.Jeevanantham, R.Venkatnarayanan. Der Pharmacia Lettre. 2010, 2(6),289-295.
3. EF. Elkady. Drug Test Anal. 2011, 3(4), 228-33.
4. WA.Nouruddin, MA.Hegazy, A.Mohamed, AA.Acta Pharm, 2012, 62,191-200.
5. GT. Boyka, PP.Ivanka, BZ.Alexander, TP.Plamen. IntJ Pharm SciRev Res. 2012, 16(1),13-16.
6. C. Sumit, A. Saahil, S.Tanvi. American Journal of PharmTech Research. 2012, 2(6)312-321.
7. WA.Nouruddin, MA. Hegazyb A, M. Abdelkawy, EA.Abdelaleem Journal of liquid chromatography and amp related technologies. 2012, 35(16), 2229-2242.
8. BW.Yogita, DP.Dipak. International Journal of Pharmacy and Pharmaceutical Sciences. 2013; 5(3):358-363.

Corresponding author N.Ramalakshmi.

C.L.Baid Metha college of Pharmacy. Chennai,

On line publication Date: 10.9.2017