Research Article

Method Development, Validation and Degradation Studies of Methocarbamol from Methocarbamol Injection

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ABSTRACT

A new simple, rapid, and sensitive reversed-phase liquid chromatographic method was developed for the estimation of methocarbamol (MC) from methocarbamol injection. The chromatographic separation was achieved on C_{18} column (phenomenex 100 x 4.6mm, 3.5µ) at ambient temperature and effluent monitored at 274 nm. The mobile phase consisted of phosphate buffer (pH 4.5) and methanol in the ratio of 70:30 v/v. The flow rate was maintained at 1 ml/min. The method was validated with respect to linearity, precision, accuracy, ruggedness, LOD, LOQ and robustness. The assay methods were found to be linear from 50-150 µg/ml. All validation parameters were within the acceptable range. Moreover, MC was subjected to different stress conditions, like acid, alkali, oxidation, and thermal degradation. The degradation studies indicated MC showed degradation in acid, alkaline, H_2O_2, thermal and in photo degradation condition. The degradation product of MC was resolved well from the pure drug with significant differences in their retention time values. This method was also successfully employed for quantitative analysis of MC from its injection formulations. The developed method is stability indicating and can be used to separate the degradants and determine the stability of samples.

Keywords: Methocarbamol Injection, RP-HPLC, Forced degradation studies.

INTRODUCTION

Methocarbamol (MC) is chemically recognized as 2-hydroxy-3-(2-methoxy phenoxy) propyl carbamate and its molecular weight is 241.24 g/mol. It is a white powder and sparingly soluble in water and chloroform, soluble in methanol (only with heating) and soluble in propylene glycol. MC (Fig: 1) is a central acting skeletal muscle relaxant, it reduces the skeletal muscle tone by selective action on the cerebro spinal axis, without altering the consciousness and may also involve in the inhibition of carbonic anhydrase. The muscle relaxant effects of methocarbamol are largely attributed to central depressant effects.

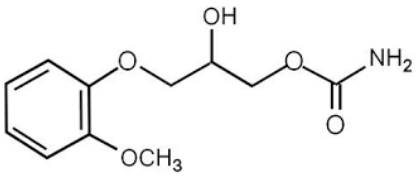

Figure 1: Structure of methocarbamol

Many literature surveys revealed that various analytical methods involving UV Spectrophotometry, [1-6] quantitation and simultaneous determination by RP-HPLC, Stability-indicating HPLC, HPTLC[7-19] has been reported for MC in single and in its combination dosage forms. Apart from this, various literature regarding determination of residual solvents in MC pure drug was determined by gas chromatography method[20, 21], RP-LC[22], isocratic SFC and chemometric method for determination of MC were reported so far.[23] In this paper, an attempt has been made to develop, validate and to study the degradation of MC for its estimation in MC injection formulation with good precision, linearity and reproducibility according to the international conference on harmonization guidelines.[24,25]

METERIALS AND METHODS

Equipments

The chromatographic technique performed on a LC 10A, VP Shimadzu with DAD detector, reversed phase C_{18} column (100x4.6mm,3.5µ) as a stationary phase, mettler toledo analytical balance, milli pore –rankem solvent filtration unit with PVDF 0.45µ filters was used in this study.

Materials

Methocarbamol injection [Robinax (100 mg/ml), (10 ml in 1 vial)] was obtained from khandelwal laboratories private limited, Mumbai. Methocarbamol (API) was obtained as gift sample. HPLC grade methanol and monobasic potassium phosphate were obtained from Merk. Orthophosphoric acid was procured from SD fine chem., Mumbai. All the chemicals used were of A.R. Grade.

Chromatographic conditions

The sample separation was achieved on a C_{18} (100x4.6mm, 3.5µ) PHENOMENEX column, aided by mobile phase mixture of buffer (pH 4.5): methanol (70:30 v/v). The flow rate was 1 ml/min with the injection volume as 5 µl at ambient temperature and was detected on UV detector at wavelength of 274 nm.

Buffer preparation

Weighed about 6.8 g of monobasic potassium phosphate dissolved in 1000 ml of Water. This solution is mixed well and adjusted the pH to 4.50 ±0.2 with dilute orthophosphoric acid (OPA) solution. Thereafter, filtered the solution through 0.45 µ membrane filter.

Mobile phase preparation

Prepared a mixture of methanol and buffer in the ratio of 30:70 v/v respectively, mixed well and sonicated for 10 min.

Standard solution preparation

Accurately weighed about 50 mg of Methocarbamol working standard into a 50 ml volumetric flask and added about 25 ml of diluent, sonicated to dissolve and dilute to volume up to 50 ml. From this 25 ml was pipette out into a 50 ml volumetric flask and the volume is made up to 50 ml to get the final concentration of 500 µg/ml.

Sample solution preparation

A number of three vials of the sample were transferred in to the clean and dry beaker. From this pipette out 5 ml of the pooled sample solution into a 100 ml volumetric flask, add 30 ml of the diluents to mix well and made to the volume with diluent and mix thoroughly. Further from this 5 ml of the solution transferred to 50 ml volumetric flask and made to the volume to 50 ml with diluent to get the concentration of 500 µg/ml.

Determination of working wavelength (λ_{max})

Weighed and transferred about 25 mg of MC working standard into a 50 ml volumetric flask, added about 10 ml of diluent to dissolve and volume is made up to 50 ml with diluent. Above solution is scanned in the range of 200 nm to 400 nm. The λ_{max} was found to be 274 nm.

RESULTS

Method Development and Validation

After the several initial trials with the mixtures of methanol, acetonitrile, OPA and buffers in various combinations and proportions, a trial with mobile phase mixture of phosphate buffer and methanol (70:30 v/v), flow rate of 1ml/min provide a sharp peak. The chromatogram was shown in Fig.2.

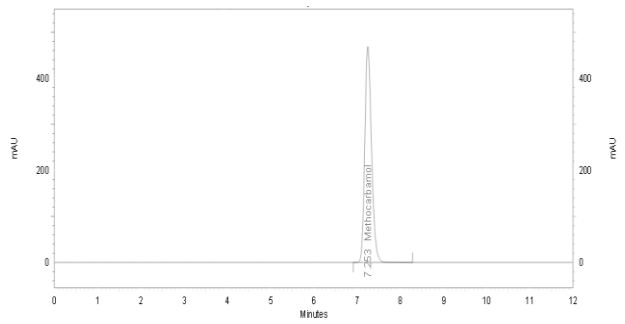

Figure 2: A typical chromatogram for Methocarbamol Linearity

A series of solutions were prepared using MC working standard at concentration levels from 50% to 150% of assay test concentration and each solution was injected into HPLC as per methodology (50%, 80%, 90%, 100%, 110%, 120%, and 150%). Calibration curve with concentration verses peak area was plotted and the obtained data were subjected to regression analysis using the least square method. (Fig: 3)

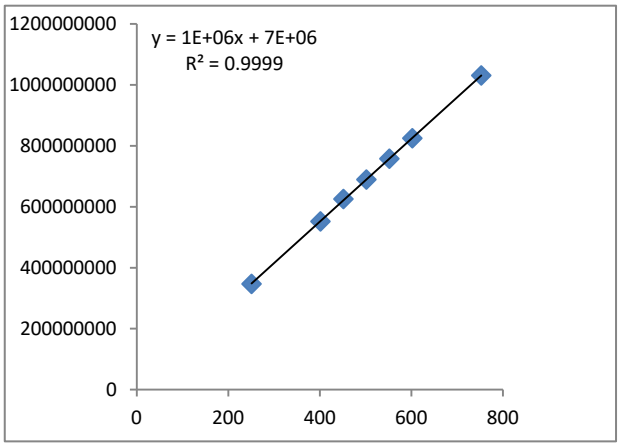

Figure 3: Linearity plot

Precision

System precision

Standard solution was prepared as per test method and injected six times in to chromatographic system. (Table 1)

Method precision

Prepared six sample solution as per test method and injected each solution into chromatographic system. (Table 2)

Table 1: System precision for Methocarbamol

Injection	Methocarbamol peak area
1	692066197
2	692421636
3	691842369
4	692405761
5	691341845
6	693140784
Mean	**692203099**
SD	**609707**
%RSD	**0.1**

Accuracy (Recovery)

A study of accuracy was conducted. Drug assay was performed in triplicate as per test method by spiking the MC drug substance to the placebo equivalent to 80%, 100%, and 120% of the labeled amount as per the test method. The average % recovery of MC was calculated. Separately inject the blank, placebo, MC in to the chromatograph. (Table 3)

Table 2: Method precision for Methocarbamol

Sample No.	% Assay (mg/ml)
1	100.19
2	100.25
3	100.42
4	100.47
5	100.45
6	100.26
Mean	**100.32**
Standard deviation	**0.11**
%RSD	**0.1**

Ruggedness

Ruggedness of the test method was studied by analyzing the sample by two analysts. The % RSD of the assay values between the two analysts was calculated. (Table 4)

Robustness

To demonstrate the robustness of the method, prepared solution as per test method were injected at different variable conditions like flow rate, wavelength and mobile phase. (Table 5, 6 & 7)

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve. The LOD and LOQ were found to be 10.127 µg/ml and 30.687 µg/ml respectively.

Table 3: Accuracy data for methocarbamol

Spiked level	Amount added (in mg)	Amount found (in mg)	% Recovery	Mean	SD	% RSD
80% Sample 1	401.62	397.97	99.1	99.1	0.06	0.1
80% Sample 2	401.57	398.36	99.2			
80% Sample 3	401.38	397.95	99.1			
100% Sample 1	501.87	500.63	99.8	99.7	0.10	0.1
100% Sample 2	501.99	500.46	99.7			
100% Sample 3	501.71	499.95	99.6			
120% Sample 1	602.72	598.99	99.4	99.4	0.06	0.1
120% Sample 2	602.23	599.17	99.5			
120% Sample 3	602.35	598.81	99.4			

Table 4: Results for Ruggedness

S.No	Analyst	Average % assay of six samples	% RSD
1	Analyst -1	100.03	0.1
2	Analyst -2	100.67	0.4

Table 5: Results for mobile phase variation study

Validation parameter	Mobile phase composition (Methanol: Buffer)	Area of Methocarbamol	% RSD of peak area	Tailing factor
1.	28:72	695351486	0.05	1.2
2.	30:70	687849851	0.12	1.1
3.	32:68	691665336	0.05	1.2

Table 6: Results for flow rate variation study

Validation parameter	Flow rate	Area of Methocarbamol	% RSD of the peak area	Tailing factor
1	1.1 ml/min	84305311	0.02	1.2
2	1.0 ml/min	94627550	0.13	1.1
3	0.9 ml/min	103149994	0.06	1.1

Table 7: Results for wavelength variation study

Validation parameter	Wavelength in nm	Area of Methocarbamol	% RSD of peak area	Tailing factor
1	276	659608867	0.05	1.2
2	274	694442981	0.004	1.2
3	272	692833536	0.06	1.2

Forced Degradation Studies

Stability studies were carried out to provide evidence on how the quality of drug varies under the influence of a variety of environmental conditions like acid, alkali hydrolysis, oxidation, temperature, photo degradation condition, etc. (Table 8)

Acid degradation

To the sample solution of MC, 2 ml of 1N HCL was added and this solution is kept at 80°C on water bath for 30 min and cooled to room temperature and the sample solution is neutralized with NaOH and finally made to the volume with diluent and centrifuged, from the above solution 25 ml was taken into 50 ml volumetric flask diluted to volume. A placebo also prepared in the same manner and analyzed by proposed HPLC method. The representative chromatogram was shown in Fig.4.

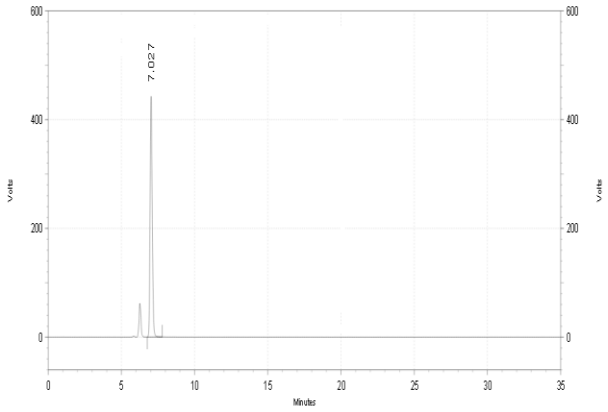

Figure 4: Representative chromatogram for acid degradation

Alkali degradation

To the sample solution of MC, 2 ml of 0.1N NaOH was added and this solution is kept at 80°C on water bath for 30 min and cooled to room temperature and the sample solution is neutralized with HCL and finally made to the volume with diluent and centrifuged, from the above solution 25 ml was taken into 50 ml volumetric flask diluted to volume, placebo also prepared in the same manner and analysed by proposed HPLC method. The representative chromatogram was shown in Fig. 5.

Oxidative degradation

To the sample solution of MC, 2 ml of 3% H_2O_2 was added and this solution is kept at room temperature for 30 min and 80°C on water bath for 30 min and cooled to room temperature and centrifuged, from the above solution 25 ml was taken into 50 ml volumetric flask diluted to volume, placebo also prepared in the same manner and analyzed by proposed HPLC method. The representative chromatogram was shown in Fig.6.

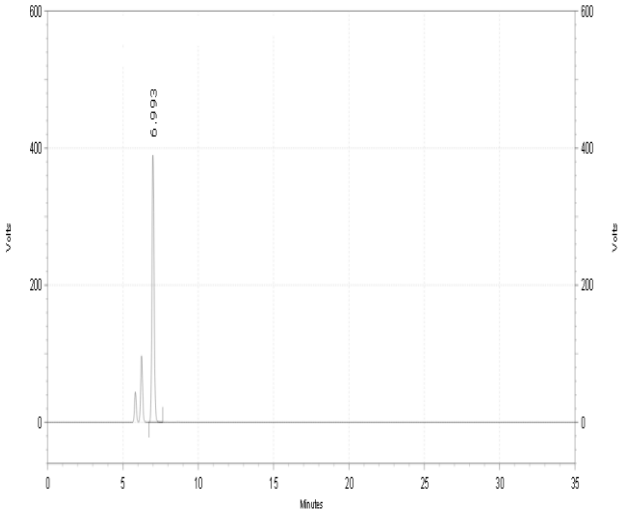

Figure 5: Representative chromatogram for alkali degradation

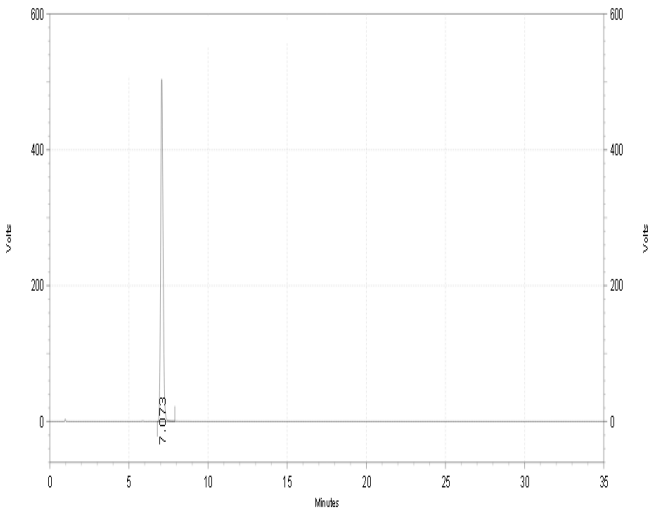

Figure 6: Representative chromatogram for oxidative degradation

Thermal degradation

Sample solution of MC was exposed into dry heat at 50°C for 120 hours and cooled to room temperature, the resulting solution was centrifuged, 25 ml of this solution was taken into 50 ml of volumetric flask diluted to volume, placebo also prepared in the same manner and

analyzed by proposed HPLC method. The chromatogram was recorded and shown in Fig.7.

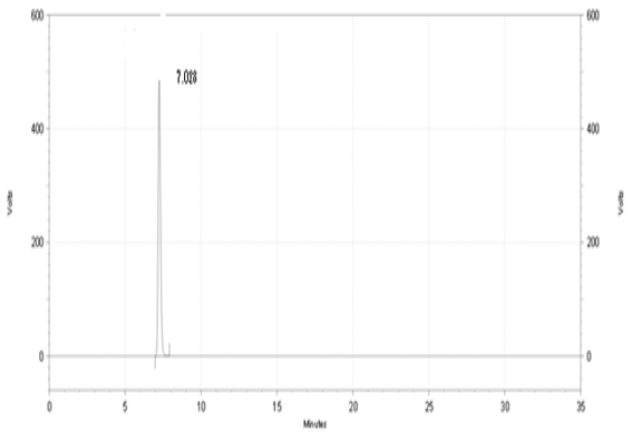

Figure 7: Representative chromatogram for thermal degradation

Photo degradation

The sample solution of MC was exposed to white the white fluorescent light for, 1.2 million LUX hours and UV light 200 watt-hours/meter square and the resulting solution is centrifuged, 25 ml of this solution was taken into 50 ml of volumetric flask diluted to volume, placebo also prepared in the same manner and analyzed by proposed HPLC method. The chromatogram was recorded and was shown in Fig. 8.

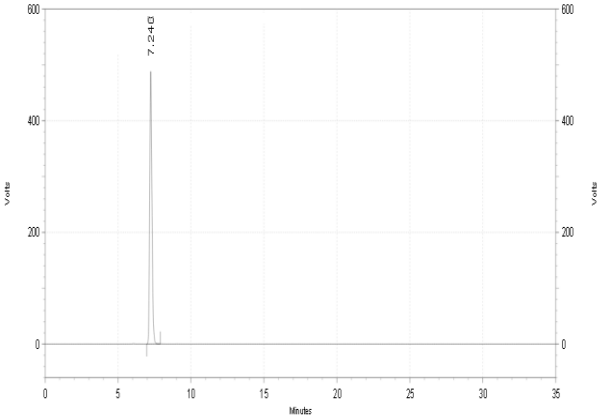

Figure 8: Representative chromatogram for photo degradation

Table 8: Forced degradation studies

Condition	% assay	% degradation
Acid degradation	87.8	12.2
Base degradation	80.81	19.19
Oxidative degradation	84.38	15.62
Thermal degradation	102.01	Nil
Photolytic degradation	102.19	Nil

DISCUSSION

A simple Reverse phase high performance liquid chromatographic method has been developed and subsequently validated for estimation of methocarbamol from methocarbamol injection. The separation was carried out by using a methanol: buffer (30:70 v/v). The detection was carried out at 274 nm. The column was phenomenex C_{18} packed column (100 x4.6mm 3.5µm). The flow rate was selected as 1.0 ml/min. The retention time of methocarbamol was found be 7.253 min. The asymmetry factor or tailing factor of MC was found to be 1.1, which indicates symmetrical nature of the peak. The number of theoretical plates of MC was found to be 10563, which indicates the efficient performance of the column. These parameters represent the specificity of the method.

From the linearity studies, specified concentration levels were determined. It was observed that MC was linear in the range of 50 % to 150 % for the target concentration by RP-HPLC. The linearity range of MC 50 % to 150 % was found to obey linearity with a correlation coefficient to 0.9999. The validation of the proposed method was verified by system precision and method precision by RP-HPLC. The %RSD of system suitability for methocarbamol was found to be 0.1. The validation of the proposed method was verified by recovery studies. The percentage recovery range was found to be satisfied which represented in results. The robustness studies were performed by changing the mobile phase, flow rate and wavelength. The ruggedness study was also performed.

MC was subjected to forced degradation studies and results showed that the molecule undergoes degradation under basic, acidic and oxidative degradation conditions. Statistical analysis proves that the method is precise, selective and accurate for the estimation of MC in its injection dosage form. As the method separates the drug from its degradation products, it can be employed as a stability indicating one. Hence it is concluded that the developed method could also applicable in quality control testing in bulk drug manufacturing and analysis of pharmaceutical dosage form.

CONCLUSION

From the above experimental results and parameter's it was concluded that, the developed RP-HPLC method is accurate, precise, robust and also the method is cost effective and less time consuming. The forced degradation studies showed that the molecule undergoes degradation under acid, base, oxidative degradation. It can successfully applied for the estimation of methocarbamol in quality control testing, in bulk drug manufacturing, analysis of pharmaceutical dosage form and also in process testing.

REFERENCES

1. Priyanka M, Pritosh Patnayak, Arabinda patnayak, Subrahmaniyam KV, A validated stability indicating HPLC assay method for methocarbamol in bulk formulation, IJMN 1(3), 2014, 112-116.
2. Nagamani M, Ramana H,Bhadru B and Vasanthi R, Method development and validation of methocarbamol in bulk and in its formulation by UV Spectoscopy, IJPCBS, 5(1), 2013, 258-261.
3. Prabhu.J, Deshmuki Hafza, Candha.S and Prandya, Simultaneous HPLC determination of methocarbamol, paracetamol and diclofenac sodium, E-Journal of chemistry, 8(4), 2012, 1620-1625.
4. Nikita P Patel and Madhuri Hinge, Development and validation of RP-HPLC method for simultaneous determination of Methocarbamol and Diclofenac sodium in Injection dosage form, WJPRS, 5(4), 2016, 1833-1842.
5. Mavar JV, Manmode RS, Laddha SS, Stability indicating HPLC method for the determination of methocarbamol and nimesulide from tablet matrix, Der chemical sinica, 2(4), 2011, 81-85.
6. Ramalakshmi R, Savita,Nalini C N. and Arun kumar S, Method development and validation of methocarbamol and ibuprofen using RP-HPLC, JCBPS; Section A, 4(3), 2017, 919-929.
7. Salehi A, Mohammady F, Kazemipour M and Ansari M, Simultaneous UV-VIS spectrophotometric determination of aspirin and methocarbamol in tablets. Res. in Pharm. Sci., 7(5), 2012, 669.
8. Satheeshmanikandan TRS, Wali DC, Bariwal J, Kadam SS and Dhaneshwar SR, Simultaneous spectrophotometric estimation of ibuprofen and methocarbamol in tablet dosage form. Ind. J. Pharm. Sci., 66, 2004, 810-813.
9. Sharaf el Din M, Eid M and Zeid AM, Simultaneous determination of methocarbamol and aspirin binary mixture in their combined tablets by derivative and ratio derivatives spectrophotometry, Anal. Methods, 5, 2014, 5644.
10. Atay, O. and Orbey, T., Quantitative analysis of methocarbamol and paracetamol containing tablets by spectrophotometric methods, FABAD J.Pharm.Sci., 15, 1990, 223-230.
11. Kır, S., Şafak, C., Türeli, A. and Temizer, A., Determination of paracetamol and methocarbamol in a pharmaceutical preparation using 2nd derivative spectrophotometry, Fresenius J. Anal. Chem., 339 (4), 1991, 264-264.
12. Erk,. N., Özkan, Y., Banoğlu, E., Özkan S.A. and Şentürk, Z., Simultaneous determination of paracetamol and methocarbamol in tablets by ratio spectra derivative spectrophotometry and LC, J.Pharm.Biomed.Anal., 24(3), 2001, 469- 475.
13. Rao, G.R, Avadhanulu, A.B., Vatsa, D.K and Pantulu, A.R.R., Gas liquid chromatographic determination of paracetamol and methocarbamol in single and combined dosage forms Indian Drugs, 27, 1990, 576-580.
14. Vasudevan, M., Ravisankar, S., Ravibabu, T. and Nanjan, M.J. —Simultaneous estimation of paracetamol, methocarbamol and ibuprofen by reversed phase HPLC method Indian Drugs, 37(8), 2000, 386-389.
15. Erram, S.V. and Tipnis, H.P., —Simultaneous determination of methocarbamol and paracetamol from single and combined tablets by RP-HPLC. Indian Drugs, 30, 1993, 116-119.
16. M R Koupai-Abyazani, B Esaw, B Laviolett. Determination of methocarbamol in equine serum and urine by high-performance liquid chromatography with ultraviolet detection and atmospheric pressure ionization-mass spectrometric confirmation, J. Chromatogr. B. Biomed. Appl. 654 (2), 1994, 287-292.
17. R. S. Manmode, A. K. Dhamankar, J. V. Manwar . Stability indicating HPLC method for simultaneous determination of methocarbamol and nimesulide from tablet matrix, Der Chemica Sinica, 2 (4), 2011, 81-85.
18. Rosasco, Maria A. Ceresole, Rita, Forastieri,clara C.Segall, Adriana I. A stability-indicating high-performance liquid chromatographic method for the determination of methocarbamol in veterinary preparations.Journal of AOAC International, 92 (5), 2009, 1602-1605.
19. S. Alessi-Severini, R TCoutts, F Jamali, F M Pasutto. High performance liquid chromatographic analysis of methocarbamol enantiomers in biological fluids. Bio Info bank library, 45 (11), 1992, 2256-2260.
20. N. Jahnaviand VS. Saravanan, Method Development and Validation For The Determination Of Residual Solventsin Methocarbamol Pure Drug By HS-GC, International Journal Of Research In Pharmacy And Chemistry, 2(2), 2012, 456-466.
21. Camarasu CC, Szjits MM and Varga GB. Residual solvents in pharmaceutical products by gas chromatography headspace, J.Pharm.Biomed.Anal. 18, 1998, 623-639.
22. Weng N, Lee JW, Hulse JD. Development and validation of a high-performance liquid chromatographic method for the determination of methocarbamol in human plasma, J Chromatogr B Biomed Appl. 654(2), 1994, 287-92.
23. Ehab F Elkady. Simultaneous spectrophotometric determination of diclofenac potassium and methocarbamol in binary mixture using chemometric techniques and artificial neural networks, Drug Test Analysis. 3 (4), 2011, 228-233.
24. ICH guidelines for stability testing of new drug substances and products Q1A (R2), Geneva, Switzerland, 2004, 1-24.
25. ICH guidelines for validation of analytical procedures: text and methodology Q2 (R1), Geneva, Switzerland, 2005, 1-17.

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