Simultaneous HPLC Determination of Methocarbamol, Paracetamol and Diclofenac Sodium

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Abstract: A rapid and simple high performance liquid chromatography method was developed and validated at $\lambda=275$ nm for simultaneous determination of methocarbamol, paracetamol and diclofenac sodium (lamotrigine as an internal standard) from pharmaceutical preparation. The separation was performed using methanol: water: GAA, in the ratio of 400:600:05 (v/v) as the mobile phase. The detector response was linear in the range of 5 to 45 μg , 3.25 to 29.25 μg , 0.5 to 4.5 μg for methocarbamol, paracetamol and diclofenac sodium respectively. The percentage assay of methocarbamol, paracetamol and diclofenac sodium was found between 100, 99.76 and 99.31% respectively. The described method has the advantage of being rapid and easy hence it can be applied for routine quality control analysis.

Keywords: Methocarbamol, Paracetamol, Diclofenac sodium, RP-HPLC, Pharmaceutical formulation

Introduction

Methocarbamol^{1,2} has the molecular formula $\text{C}_{11}\text{H}_{15}\text{NO}_5$ and molecular weight 241.241 g/mol. Its chemical name is [2-hydroxy-3-(2-methoxyphenoxy)-propyl] aminoformate. Paracetamol²⁻⁴ has the molecular formula $\text{C}_8\text{H}_9\text{NO}_2$ and a molecular weight 151.17 g/mol. Chemical name of paracetamol is *N*-[4-hydroxyphenyl]ethanamide. Diclofenac sodium^{2,4,5} has molecular formula $\text{C}_{14}\text{H}_{11}\text{Cl}_2\text{NO}_2$ and molecular weight 318.14g/mol. Its chemical name is 2-[-2-(2, 6-dichlorophenyl) amino phenyl] ethanoic acid.

Methocarbamol 500 mg, paracetamol 325 mg and diclofenac sodium 50 mg in combination are available in market by brand name Robinaxol-D which is a muscle relaxant². It causes skeletal muscle relaxation by preferential block of polysynaptic spinal reflexes².

A literature survey reveals that there is no method reported so far for the simultaneous determination of these drugs in combined dosage form or any such pharmaceutical preparation

by RP-HPLC using UV/Visible detector even though the validation of individual drug and in combination with other drugs are reported⁶⁻⁹. In this communication we report a new method for simultaneous determination of methocarbamol, paracetamol and diclofenac sodium in combination tablet.

Figure 1. Structure of methocarbamol, paracetamol and diclofenac sodium

Experimental

The methocarbamol, paracetamol and diclofenac sodium and lamotrigine working standards were obtained as a gift sample from Watson Pharmaceutical Ltd, Cipla Ltd and Khandelwal Ltd, India with purity of 98.99, 99.89 and 99.99% respectively. The formulation of the tablet with combination of methocarbamol 500 mg, paracetamol 325 mg and diclofenac sodium 50 mg is available in market by brand name Robinaxol-D. All the reagents used were of chromatographic grade. All dilutions were performed in standard volumetric flask. Triple distilled water was prepared in the laboratory.

Instrumentation and chromatographic conditions

Chromatography was performed on Agelint high performance liquid chromatograph equipped with PU-1580 liquid chromatographic pump with 20 μ L loop and Agelint UV/Visible-1575 intelligent variable wavelength detector. The stationary phase is HiQ Sil C₁₈Hs column (4.6 mm ID $\times$ 250 mm L), using (400: 600: 05) methanol: water: GAA as mobile phase. The chromatographic conditions had previously been optimized to achieve the best resolution and peak shape. Detection was performed at $\lambda=275$ nm having flow rate of 1 mL/min. The typical chromatogram is shown in Figure 2.

Stock solution of methocarbamol (Solution 1)

50 mg of methocarbamol was accurately weighed, transferred into 1000 cm³ of volumetric flask and dissolved in the minimum amount of mobile phase and diluted up to the mark with mobile phase.

Stock solution of paracetamol (Solution 2)

32.5 mg of Paracetamol was accurately weighed and transferred into 100 cm³ of volumetric flask. It was dissolved in the minimum amount of mobile phase and then diluted up to the mark with mobile phase.

Stock solution of diclofenac sodium (Solution 3)

5 mg of Diclofenac sodium was accurately weighed, transferred into 100 cm³ of volumetric flask and dissolved in the minimum amount of mobile phase and then diluted up to the mark with mobile phase.

Stock solution of lamotrigine (Solution 4)

10 mg of Lamotrigine was accurately weighed, transferred into 100 cm³ of volumetric flask and was dissolved in the minimum amount of mobile phase and then diluted up to the mark

with mobile phase. From this solution 1 cm³ of solution was used for each standard solution as an internal standard.

Figure 2. Chromatogram showing separation by RP-HPLC

(1) Diclofenac sodium, (2) Paracetamol, (3) Lamotrigine and (4) Methocarbamol

Preparation of working standard solution

A varying amount of stock solutions of methocarbamol, paracetamol and diclofenac sodium were added to a series of 50 cm³ volumetric flasks. To each flask 1 cm³ of internal standard (Solution 4) was added. These mixtures were then diluted up to the mark with mobile phase which gave concentration range between 5 to 45 µg/cm³ of methocarbamol, 3.25 to 29.25 µg/cm³ of paracetamol and 0.5 to 4.50 µg/cm³ of diclofenac sodium.

Preparation of sample solution (Solution 5)

Twenty tablets were weighed accurately and average weight of one tablet was calculated. The tablets were crushed to furnish a homogeneous powder and a quantity equivalent to one tablet (1214 mg) was weighed and dissolved in a minimum quantity of methanol. This was transferred to the 1000 cm³ of standard volumetric flask with filtration by using a Whatman filter paper 41. The filter paper was washed with methanol and the washings were collected in to the standard volumetric flask. The contents of the flask were then diluted up to the mark with methanol and used as a sample solution.

Validation procedure

Linearity

Different concentrations of methocarbamol (5 to 45 µg), paracetamol (3.25 to 29.25 µg) and diclofenac sodium (0.5 to 4.5 µg) were prepared from stock solution of respective API in mobile phase. 20 µL of each solution was injected and the detector response for the different concentration was measured. The drug peak area was calculated for each concentration level and a graph was plotted of drug concentration against the peak area. The plot was linear in the range mentioned above. This experiment was performed thrice and the mean peak area response was used for calculations. The data was analyzed by linear regression least square fitting. The statistical data obtained is given in Table 1.

Table 1. Linear regression data

Regression Output	Methocarbamol	Paracetamol	Diclofenac sodium
Constant	-0.07128	-0.03356	0.13624
Standard error of Y estimate	1929.7277	1292.9234	1.86684
R squared	1	1	0.999
No. of observations	9	9	9
Degree of freedom	7	7	7
X Coefficient(S) (m)	1.69585	2.7274	0.18114
Standard error of coefficient	0	0	3.33×10^{-4}

System suitability

The coefficient of variation for peak area and retention time value for all the drugs was less than 2.00% for three replicate measurements of the same sample.

Robustness of the method

In the present work effect of flow rate was studied for the robustness of the method. The analysis was performed by using flow rate of ± 0.2 cm³/minute. The results are summarized in Table 2, 3 and 4 which shows that method is not affected by deliberate change in the flow rate.

Table 2. Effect of flow rate - methocarbamol

Flow rate, cm ³ /min	Retention time, min.	Theoretical plate	Resolution
1.00	9.05	8190.26	10.9333
0.8	9.28	8618.03	10.96
1.2	8.79	7732.27	10.71

Table 3. Effect of flow rate - paracetamol

Flow rate, cm ³ /min	Retention time, min.	Theoretical plate	Resolution
1.00	4.17	1111.12	4.5809
0.8	4.41	1244.68	4.4833
1.2	3.97	1007.01	4.6095

Table 4. Effect of flow rate - (diclofenac sodium)

Flow rate, cm ³ /min	Retention time, min.	Theoretical plate	Resolution
1.00	3.02	3656.28	13.7
0.8	3.24	4199.04	13.7
1.2	2.83	3196.05	13.76

Peak asymmetry and peak tailing

The result shows that within the concentration ranges mentioned above there was an excellent correlation between peak area ratio and concentration of the each drug. The results are given in Table 5.

Table 5. Asymmetry factor and peak tailing factor

Components	Asymmetry factor	Peak tailing factor
Methocarbamol	1	1
Paracetamol	0.5	0.75
Diclofenac Sodium	1	1
Lamotrigine	1	1

Assay (from pharmaceutical preparation)

20 µL of working standard solution of methocarbamol, paracetamol and diclofenac sodium and sample solution were injected and chromatograms were developed and evaluated. The procedure was repeated three times, individually weighing the tablet powder (average weight of one tablet) each time. The responses from the standard and sample were used to calculate the amounts of the drug in the tablet. Results obtained are shown in Table 6.

Table 6. Results of HPLC assay studies (n=3)

	Weight of sample taken, mg	Amount found, mg	SD	C.O.V%	%Assay	SD	C.O.V %
Methocarbamol	1213	500.53	0.2078	0.0415	100.1	0.0406	0.0405
Paracetamol	1212	323.42	1.5242	0.4712	99.51	0.5411	0.5437
Diclofenac Na	1211	49.75	0.4436	0.9816	99.51	0.5100	0.5125

Recovery

The accuracy of the experiment was established by using a fixed amount of pre-analyzed sample *i.e.* 2.5 cm³ of solution 5 was taken in an 50 cm³ of standard volumetric flask to this 1 cm³ internal standard lamotrigine (solution 4) was added and the contents of the flask were diluted up to the mark with mobile phase. This served as the zero level *i.e.* 100%. The results of recovery analysis are given in Table 7. The mean recovery is within acceptable limits which indicate that the method is accurate.

Table 7. Results for recovery analysis (n=3)

S.No	Formulation	Labeled amount (mg/tablet)	Amount of standard added mg/Tab	Amount found (mg/tablet)	Percentage recovery
1	Methocarbamol	500	1.25	1.25	100
2	Paracetamol	325	0.814	0.814	100
3	Diclofenac Na	50	0.122	0.122	100

Each reading is the mean of the three readings

Determination of reliability of the assay method

Statistical parameters namely standard deviation (S.D) and percentage coefficient of variation (C.O.V. %) were calculated to study the reliability of the method. Low values of these statistical parameters for recovery indicates high reliability of the method, these values are given in Table 8.

Table 8. Statistical parameters

	Level	Amount found mg/tablet	Standard deviation	C.O.V.%
Methocarbamol	0.0	1.25	0.00	0.00
Paracetamol	0.0	0.814	0.0003	0.0368
Diclofenac Na	0.0	0.122	0.00	0.00

Results and Discussion

Use of methanol: water: GAA in volume ratio of 400: 600: 05 resulted in good separation of the drugs with no interfering peak at the retention time of the methocarbamol, paracetamol and diclofenac sodium and lamotrigine *viz.* 9.00 minute, 4.15 minute, 3.00 minute and 5.75 minute respectively as shown in Figure 2.

Figure 2 shows typical chromatogram obtained from methocarbamol, paracetamol and diclofenac sodium. Regression analysis for the calibration data for all the drugs showed that

the dependent variable (peak area) and independent variable (concentration) were represented by the equation $y=1.69585x + 0.07128$ for methocarbamol, $y=0.36401x - 38.37153$ for paracetamol, $y=0.18114x + 0.13624$ for diclofenac sodium.

The correlation coefficient obtained was 1.00, 1.00 and 0.999 for methocarbamol, paracetamol and diclofenac sodium respectively. It shows the good linear relationship between the concentration range 5 to 45 μg , 3.25 to 29.25 μg and 0.5 to 4.5 μg for methocarbamol, paracetamol and diclofenac sodium respectively.

The coefficient of variation for peak area and retention time value for all the drugs was less than 2% for three replicate measurements of the same sample. This showed that the method and the system are suitable for determination of methocarbamol, paracetamol and diclofenac sodium.

The assay of methocarbamol, paracetamol and diclofenac sodium was found to be 100.10, 99.51 and 99.51% respectively. The recovery of methocarbamol, paracetamol, and diclofenac sodium was found to be 100, 100 and 100% respectively from recovery studies which indicates high accuracy of the method.

Conclusion

As the proposed method is highly accurate, selective and precise hence can be used for a routine quality control analysis and quantitative simultaneous determination of methocarbamol, paracetamol and diclofenac sodium in pharmaceutical preparations. The method is also fast and requires approximately 10 minute for analysis.

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