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ORIGINAL PAPER

Spectrofluorometric Determination of Methocarbamol in Pharmaceutical Preparations and Human Plasma

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Abstract A simple, sensitive and rapid spectrofluorometric method for determination of methocarbamol in pharmaceutical formulations and spiked human plasma has been developed. The proposed method is based on the measurement of the native fluorescence of methocarbamol in methanol at 313 nm after excitation at 277 nm. The relative fluorescence intensity-concentration plot was rectilinear over the range of 0.05–2.0 μg/mL, with good correlation (r=0.9999), limit of detection of 0.007 μg/ mL and a lower limit of quantification of 0.022 μg/ mL. The described method was successfully applied for the determination of methocarbamol in its tablets without interference from co-formulated drugs, such as aspirin, diclofenac, paracetamol and ibuprofen, The results obtained were in good agreement with those obtained using the official method (USP 30).The high sensitivity of the method allowed the determination of the studied drug in spiked human plasma with average percentage recovery of 99.42±3.84.

Keywords Spectrofluorometry · Methocarbamol · Native fluorescence and spiked plasma

Introduction

Methocarbamol or guaifenesin carbamate (Fig. 1) [3-(2-methoxyphenoxy)-1,2-propanediol 1-carbamate] [1], is a central muscle relaxant that is used to treat skeletal muscle spasms [2]. The United States Pharmacopoeia [3] recommends a spectrophotometric method for the determination of methocarbamol raw material and HPLC method for its tablets. Several analytical methods have been reported for the determination of methocarbamol in tablets or in human plasma like, Spectrophotometry [4–9], H^1-NMR Spectroscopy [10], TLC [11], GC [12–14], HPLC [5, 15–27] and supercritical fluid chromatography [28–30].

Methocarbamol, like other muscle relaxants is co-formulated with analgesics to relieve the pain associated with the muscle spasm, such as aspirin, diclofenac potassium, paracetamol and ibuprofen. To solve the problem of the interferance likely to be encountered from such co-formulated drugs, most reported methods used derivative spectrophotometry or chromatographic methods. Reviewing the literature up to the present, reveals that nothing has been reported concerning the spectrofluorometric determination of methocarbamol. This encouraged us to study the native fluorescence of the drug in an attempt to develop a simple and sensitive spectrofluorometric method for its determination, either alone or in its co-formulated preparations. The method was extended to the in-vitro determination of the drug in spiked human samples, the results obtained were promising.

Experimental

Apparatus

*The fluorescence spectra and measurements were recorded using a Perkin-Elmer UK model LS 45 luminescence spectrometer, equipped with a 150 Watt Xenon arc lamp, the excitation and emission wavelengths were 277 and 313 nm respectively. Slit width for both monochromators were set at 10 nm, and the

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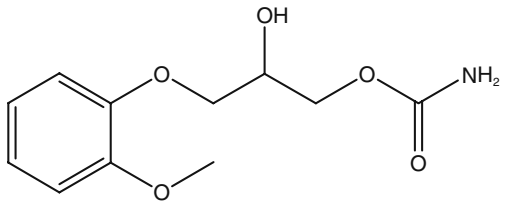

Fig. 1 Structural formula of methocarbamol

photomultiplier voltage was set to auto. Quartz cell 1 cm was used.
*A Consort NV P901 pH Meter calibrated with standard buffers was used for pH measurements.

Materials and Reagents

* All chemicals were of analytical grade.

- Methanol was obtained from Sigma-Aldrich (Germany).
- Sodium carbonate and chloroform were obtained from El- Nasr Chem. Co. (Cairo.Egypt).
- Plasma was kindly provided by Mansoura University Hospital, Mansoura, Egypt and stored at 4°C until use after gentle thawing.
- Methocarbamol, pure sample 99.5% (batch # TR080111) was kindly provided by Sigma Pharmaceutical Company, Cairo, Egypt, and was used as received.
- Ibuflex® tablets (labeled to contain 750 mg methocarbamol and 400 mg ibuprofen/tablet), Methorelax® tablets (labeled to contain 400 mg methocarbamol and 325 mg paracetamol/tablet), Dimra® tablets (labeled to contain 500 mg methocarbamol and 50 mg diclofenac potassium/tablet), Laboratory prepared tablets (500 mg of methocarbamol, 20 mg talc powder, 15 mg maize starch, 15 mg lactose and 100 mg magnesium stearate per tablet), Laboratory prepared tablets (400 mg methocarbamol , 325 mg aspirin, 20 mg talc powder, 15 mg maize starch, 15 mg lactose and 100 mg magnesium stearate per tablet).

Standard Solution

Stock solution of 100.0 μg/mL of methocarbamol was prepared in methanol. The solution was found to be stable for at least 1 week when kept in the refrigerator. A working standard solution of 10.0 μg/mLof the drug was prepared by further dilution with methanol as appropriate.

Procedures for Calibration Graph

Transfer accurately measured aliquots of the working solution so that the final concentration is in the range of 0.05–2 μg/mL into a series of 10 mL volumetric flasks and completed to the volume with methanol. The fluorescence intensity was measured at 313 nm after excitation at 277 nm. The relative fluorescence intensity was plotted against the final concentration of the drug. Alternatively the corresponding regression equation was derived.

Procedures for Tablets

- An accurately weighed quantity of 10 pulverized tablets equivalent to 10 mg of the drug were transferred into 100 mL volumetric flask and completed to the volume with methanol, sonication for 30 min and filtered, and the above procedure was followed. The nominal contents were calculated either from previously plotted calibration graph or using the corresponding regression equation.

Procedures for Spiked Human Plasma Transfere 1 mL aliquots of plasma into centrifuge tubes and, spike with methanolic solution of methocarbamol in different concentrations. Add 1 mL of saturated solution of sodium carbonate, mix and centrifuge for 20 min at 5,000 rpm. Add 5 mL of chloroform and centrifuged as previous. The upper aqueous layer was discarded and collect the chloroformic layer and evaporate to dryness [31]. Redissolve the residue in methanol and complete to 10 ml in volumetric flasks and complete as described under the calibration graph.

Results and Discussion

Methocarbamol methanolic solution was found to exhibit an intense native fluorescence at 313 nm after excitation at 277 nm (Fig. 2). Different solvents and

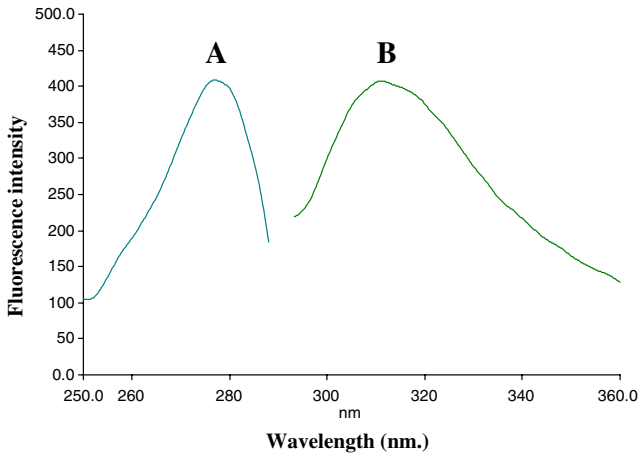

Fig. 2 **a** Excitation and **b** Emission spectra of (1 μg/mL) methocarbamol in methanol

media were tried such as water, methanol, acetonitrile, acetone, dimethylsulfoxide(DMSO), dimethylformamide (DMF), 0.1 M HCl, 0.1 M NaOH, acetate buffer(pH 4.5), borate buffer(pH 7.5, 9.5) , the pH has no effect or may decrease the fluorescence intensity of the drug so no buffer was used throughout the work as shown in Table 1 and the maximum fluorescence intensity was obtained in methanol, hence it was recommended throughout the work.

The effect of different surfactants and sensitizers on the fluorescence intensity of methocarbamol was studied, Thus 0.5% SDS, 0.1% cetrimide, 0.1% methyl cellulose, 0.5% β-cyclodextrin and 1% tween 80 were studied by adding 1 mL of each surfactant to the methanolic solution of the drug(final concentration 0.5 μg/mL) . It is obvious from the results (Fig. 3) that the presence of surfactants resulted in no significant effect or may decreased fluorescence intensity as in case of tween 80. Therefore, no surfactant was used in this work.

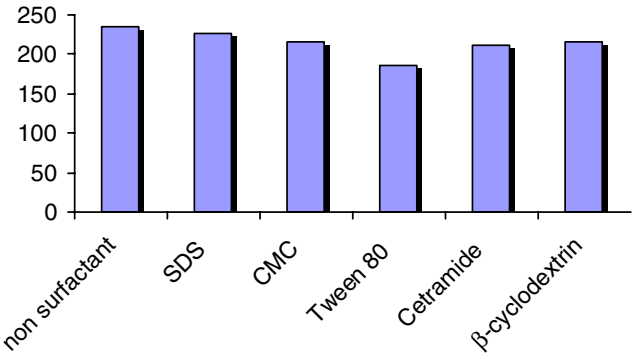

Fig. 3 Effect of various surfactants on the fluorescence intensity of methocarbamol (0.5 μg/mL) in methanol

Validation of the Method

The validity of the method was checked by testing linearity, specificity, accuracy, repeatability and precision according to ICH recommendations [32].

Linearity

Using the above procedures it was found that, there is a linear relationship between RFI and concentrations over the range of 0.05–2 μg/mL. Linear regression analysis of the data gave the following equation:

$$RFI = 9.75 + 392\ C$$

Where RFI is the relative fluorescence intensity and C is the final concentration of methocarbamol in μg/mL. Statistical analysis of the data gave small values of the standard deviation of residual (S*y/x*), the standard deviation of the intercept (S*a*), the standard deviation of the slope (S*b*) and the percentage of relative error(% Er) are all shown in Table 2.

Limit of Quantification and Limit of Detection

The limit of quantification (LoQ) was determined by establishing the weakest concentration that can be measured, below which the calibration graph is non linear, it was found to be 0.022 μg/mL. The limit of detection (LoD) was determined by evaluating the weakest concentration that can be detected and was found to be 0.007 μg/mL.

Table 1 The effect of different solvents and buffers on the fluorescence intensity of 0.5 μg/mL of methocarbamol

Solvent	Fluorescence Intensity
Water	215
Methanol	236
Acetonitrile	238 (with very highblank reading
Acetone	No Fluorescence
Dimethylformamide(DMF)	No Fluorescence
Dimethylsulfoxide(DMSO)	132
0.1 N NaOH	166
0.1 N HCl	187
Acetate buffer (pH 4.5)	182
Borate buffer (pH 7.5)	184
Borate buffer (pH 9.5)	196

Table 2 Analytical performance data for the spectrofluorometric determination of methocarbamol

Parameter	Value
Wavelength (λex. / λem.) (nm)	277 / 313
Linearity range (μg/mL)	0.05–2
Intercept (*a*)	9.75
Slope (*b*)	392
Correlation coefficient (*r*)	0.9999
S.D. of residuals (S*y/x*)	1.62
S.D. of intercept (S*a*)	0.90
S.D. of slope (S*b*)	0.87
% RSD	1.28
% Error	0.48
Limit of detection (LoD) (μg/mL)	0.007
Limit of quantification (LoQ) (μg/mL)	0.022

* **Where**: Sy/x : standard deviation of the residuals; S_b: standard deviation of the slope, Sa, standard deviation of the intercept, %Error = % RSD/√n

Table 3 Application of spectrofluorometric method to the determination of the methocarbamol in its raw material

Parameter	Proposed method			Reference method [3]	
	Conc. added (μg/mL)	Conc. found (μg/mL)	% Found	Conc. added (μg/mL)	% Found
Methocarbamol	0.05	0.051	102.00	20	101.50
	0.1	0.098	98.00	40	100.75
	0.2	0.202	101.00	50	101.96
	0.5	0.496	99.20		
	1	1.000	100.00		
	1.5	1.506	100.40		
	2	1.996	99.80		
$\overline{X} \pm SD$	100.05±1.28			101.40±0.61	
t	1.69 (2.30)				
F	4.38 (19.32)				

Each result is the average of three separate assays.

Values between brackets are the tabulated t and F values at $p=0.05$

Table 4 Assay results for the spectrofluorometric determination of methocarbamol in synthetic mixtures with aspirin, ibuprofen, dicofenac and paracetamol

Parameter	Amount taken (μg/mL)		Amount found of methocarbamol (μg/mL)	% Found
	Methocarbamol	Aspirin		
	0.25	0.2	0.2521	100.85
	0.615	0.5	0.6079	98.87
	1.23	1	1.2218	99.35
$\overline{X} \pm SD$				99.69±1.03
%RSD				1.03
%Error				0.59
	Methocarbamol	Ibuprofen		
	0.375	0.2	0.3696	98.55
	0.94	0.5	0.9327	99.23
	1.87	1	1.8958	101.38
$\overline{X} \pm SD$				99.72±1.47
%RSD				1.47
%Error				0.84
	Methocarbamol	Diclofenac		
	0.5	0.05	0.5093	101.86
	1	0.1	1.0137	101.37
	1.5	0.15	1.4869	99.13
$\overline{X} \pm SD$				100.78±1.45
%RSD				1.45
%Error				0.83
	Methocarbamol	Paracetamol		
	0.25	0.2	0.2468	98.71
	0.615	0.5	0.6080	98.86
	1.23	1	1.2229	99.43
$\overline{X} \pm SD$				99.00±0.37
%RSD				0.37
%Error				0.21

Table 5 Application of fluorimetric method to the determination of the methocarbamol in pharmaceutical preparations

Preparation	Proposed method			Reference method [3]	
	Conc. added (μg/mL)	Conc. found (μg/mL)	Recovery (%)	Conc.added (μg/mL)	Recovery (%)
Prepared tablets (500 mg methocarbamol/tablet)	0.5	0.5037	100.74	50	101.34
	1	0.9931	99.31	80	100.55
	1.5	1.4890	99.27	100	100.26
$\overline{X} \pm SD$	99.77±0.83			100.71±0.55	
t	1.62 (2.77)				
F	2.24 (19)				
Prepared tablets(400 mg methocarbamol+325 mg aspirin/tablet)	0.5	0.4918	98.37	50	99.11
	1	0.9874	98.74	80	101.18
	1.5	1.5091	100.61	100	99.71
$\overline{X} \pm SD$	99.24±1.20			100.00±1.06	
t	0.82 (2.77)				
F	1.27(19)				
Ibuflex® tablets (750 mg methocarbamol+400 mg ibuprofen/tablet)	0.5	0.4971	99.42	50	99.50
	1	1.0143	101.43	80	100.50
	1.5	1.4905	99.37	100	99.23
$\overline{X} \pm SD$	100.07±1.17			99.74±0.66	
t	0.42 (2.77)				
F	3.08 (19)				
Dimra® tablets (500 mg methocarbamol+50 mg diclofenac potassium /tablet)	0.5	0.4947	98.95	50	99.88
	1	0.9926	99.26	80	99.70
	1.5	1.5022	100.15	100	101.13
$\overline{X} \pm SD$	99.45 ±0.62			100.23±0.77	
t	1.36 (2.77)				
F	1.56 (19)				
Methorelax®tablets(400 mg methocarbamol+325 mg Paracetamol/tablet)	0.5	0.4927	98.54	50	100.71
	1	0.9917	99.17	80	99.80
	1.5	1.5282	101.88	100	101.25
$\overline{X} \pm SD$	99.86 ±1.77			100.58±0.73	
t	0.65 (2.77)				
F	5.86 (19)				

Each result is the average of three separate assays.

Values between brackets are the tabulated t and F values at p=0.05

Table 6 Accuracy and precision data for the determination of methocarbamol by the spectrofluorometric method

Parameter	Intra- day precision			Inter- day precision		
	Concentration added (μg/ml)	Concentration found (μg/ml)	% Found	Concentration added (μg/ml)	Concentration found (μg/ml)	% Found
Data	0.2	0.201	100.50	0.2	0.203	101.50
	0.5	0.497	99.40	0.5	0.501	100.20
	1.0	0.997	99.70	1.0	1.003	100.30
$\overline{X} \pm SD$	99.86±0.57			100.66±0.72		
%RSD	0.57			0.72		
%Error	0.33			0.42		

N.B. Each result is the average of three separate determinations

Table 7 Effect of co-formulated drugs on the fluorometric determination of 0.5 μg/mL methocarbamol

Drug	Tolerance limit (μg/mL)
Aspirin	1.1
Paracetamol	1.0
Diclofenac	1.4
Ibuprofen	1.3

Table 8 Application of spectrofluorometric method to the determination of the methocarbamol in spiked plasma

Parameter	Conc. added (μg/ml)	Conc. found (μg/ml)	% Recovery
	0.2	0.210	105.00
	0.5	0.481	96.20
	0.8	0.788	98.50
	1.0	0.980	98.00
Mean±SD			99.42±3.84

N.B. Each result is the average of three separate determinations

Accuracy and Precision

To prove the accuracy of the proposed method, the results of the assay of the studied drug in pure form and pharmaceutical preparations were compared with the reference method [3]. Statistical analysis [33] of the results obtained by the proposed and reference method using student's *t*-test and variance ratio *F*-test showed no significant differences between them regarding accuracy and precision, Tables 3, 4 and 5.

Intraday day and interday precisions were assessed using three concentrations and three replicates of each concentration, the relative standard deviations were found to be very small indicating reasonable repeatability of the proposed method as shown in Table 6.

Specificity

The specificity of the proposed method was proven by its ability to determine methocarbamol in its tablets without interference from the common excipients, as shown in Table 5.The interference likely to be introduced from the co-formulated drugs, such as aspirin, paracetamol, diclofenac and ibuprofen were studied under the same experimental conditions by calculation of the tolerance limit for each drug (concentration of interfering drug causing less than 3% relative error), as shown in Table 7.

Applications of the Method

Analysis of Methocarbamol in Synthetic Mixtures

The proposed method was successfully applied to the analysis of methocarbamol in synthetic mixtures in different concentrations of methocarbamol in ratios 1.23: 1 for paracetamol and aspirin, 10: 1 for diclofenac and 1.87: 1 for ibuprofen, keeping the ratios as tablets, the results obtained were in good agreement with these obtained by the official HPLC method [3], as shown in Table 4.

Analysis of Dosage Forms

The proposed method was successfully applied to the analysis of methocarbamol in its tablets, the results obtained were in good agreement with these obtained by the official HPLC method [3], as shown in Table 5.

Application of the Method in Spiked Human Plasma

The high sensitivity of the proposed method allows the determination of methocarbamol in spiked human plasma (Table 8). The calibration was performed in spiked plasma, showing linearity:

$$RF = 381.3C - 20.56 \quad (r = 0.9993)$$

Where RF is relative fluorescence intensity and C is the concentration of methocarbamol in plasma in μg/mL.

Conclusion

A simple, rapid and sensitive spectrofluorometric method was developed for determination of methocarbamol. The spectrofluorometric technique, by virtue of its high sensitivity, was not used before for the determination of methocarbamol. Additionally, the simplicity of the method allows the successful determination of the studied drug in its tablets either alone or in presence of co-formulated drugs without prior separation, as well as the successful determination of the drug in spiked human plasma.

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