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A rapid spectrophotometric method for the determination of mefloquine hydrochloride

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Abstract

A simple ultraviolet spectrophotometric method for the estimation of mefloquine hydrochloride in methanol (ME_2) has been developed and has been compared with the reported ultraviolet spectrophotometric method in 0.1 N hydrochloric acid (ME_1). Analytical parameters such as stability, selectivity, accuracy and precision have been established for both the methods and evaluated statistically to assess the application of the individual methods. Both the methods were compared with the existing pharmacopoeial method for estimation of the drug. Both the methods were found to have the advantages for simplicity, stability, sensitivity, reproducibility and accuracy for using as an alternate to the existing non-spectrophotometric methods for the routine analysis of the drug in pharmaceutical formulations and also in pharmaceutical investigations involving mefloquine hydrochloride.

Keywords: Ultraviolet spectrophotometry; Mefloquine; Antimalarial

1. Introduction

The recent resurgence of malaria has renewed interest in hitherto lesser studied antimalarial agents. One such therapeutic entity is mefloquine hydrochloride, (+)erythro-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinolinemethanol hydrochloride, a blood schizonticide used for combating drug-resistant-falciparum malaria. The drug is official in the European Pharmacopoeia supplement 2001 and the official method of estimation is titrimetry [1]. Various methods have been reported for its determination out of which high pressure liquid chromatography [2–12], gas–liquid chromatography [13,14], gas chromatography [15–17], thin-layer chromatography [18], fluorimetry [19] and capillary zone electrophoresis [20] are some important methods. Even though an ultraviolet spectroscopic method [21] (ME_1) has been suggested as a method of analysis, no other analytical parameter except absorptivity has so far been reported. In this study, a simple, ultraviolet spectrophotometric method for the determination of mefloquine hydrochloride in methanol (ME_2) has been reported. Analytical parameters for the method ME_1 have also been established

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and compared with those established for the method ME_2.

2. Experimental

2.1. Apparatus

Hitachi U-2000 UV visible spectrophotometer with quartz cells of 10-mm path length; Bath sonicator (Model 120W, Vibronics Pvt. Ltd, Mumbai), Mettler Toledo DL 67 Titrator (Mettler Toledo, Switzerland), 589 Blue ribbon S & S filter paper circles of diameter 125 mm (Schleider and Schuell GmbH, Germany).

2.2. Materials

Mefloquine hydrochloride (Pharmaceutical grade) was kindly gifted by Sun Pharmaceutical Advanced Research Centre (SPARC), Baroda and was used without any further purification; methanol, anhydrous formic acid, acetic anhydride, perchloric acid and hydrochloric acid of analytical grade (S.D. Fine Chem Ltd, Boisar); 0.1 N hydrochloric acid (prepared as per the method given in the Indian Pharmacopoeia [22]); stock solutions of mefloquine hydrochloride (i) SS_1 (10 μg ml^{-1}) prepared by dissolving 20 mg of mefloquine hydrochloride in 100 ml of 0.1 N hydrochloric acid using a bath sonicator and diluting 5 ml of this solution to 100 ml with 0.1 N hydrochloric acid (ii) SS_2 (1 mg ml^{-1}) prepared by dissolving 10 mg of mefloquine hydrochloride in 10 ml of methanol.

2.3. Preparation of calibration curve

2.3.1. For ME_1

Suitable aliquots (1–10 ml) of the stock solution SS_1 were pipetted into 10 ml volumetric flasks and the volume was made upto 10 ml with 0.1 N hydrochloric acid. The solutions were shaken well for proper mixing and their absorbance measured at 222 nm.

2.3.2. For ME_2

Suitable aliquots (0.1–1.0 ml) of the stock solution SS_2 were pipetted into 10 ml volumetric flasks. The volume was made up with methanol, shaken well and the absorbance was measured at 283 nm.

The above procedure for both the methods was repeated six times. Mean absorbance values along with the regressed values (method of least squares) and statistical data for the methods ME_1 and ME_2 are shown in Tables 1 and 2, respec-

Table 1
Mean absorbance values, regressed values and statistical data of the calibration curve for the estimation of mefloquine hydrochloride in 0.1 N hydrochloric acid (ME_1)

Concentration (μg ml^{-1})	Mean ABS[a] ($\pm$ S.E.)	Regressed values[b]
1	0.116 ± 0.011	0.122
2	0.220 ± 0.022	0.226
3	0.329 ± 0.014	0.324
4	0.442 ± 0.025	0.423
5	0.619 ± 0.029	0.619
8	0.819 ± 0.029	0.815
10	1.002 ± 0.023	1.012

Regression equation^{++} statistical data. Intercept (a) = 2.974×10^{-2}; slope (b) = 9.82×10^{-2}; correlation coefficient = 0.999; $^{++}$, $n = 42$.

[a] Mean of six values.

[b] Using regression equation.

Table 2
Mean absorbance values, regressed values and statistical data of the calibration curve for the estimation of mefloquine hydrochloride in methanol (ME_2)

Concentrations (μg ml^{-1})	Mean ABS[a] ($\pm$ S.E.)	Regressed values[b]
10	0.155 ± 0.009	0.166
20	0.293 ± 0.017	0.303
30	0.459 ± 0.030	0.441
40	0.585 ± 0.028	0.579
80	1.135 ± 0.057	1.129
100	1.396 ± 0.072	1.405

Regression equation^{++} statistical data; intercept (a) = 2.785×10^{-2}; slope (b) = 1.377×10^{-2}; correlation coefficient = 0.999; $^{++}$, $n = 36$.

[a] Mean of six values.

[b] Using regression equation.

Table 3
Optical characteristics for mefloquine hydrochloride in 0.1 N hydrochloric acid and methanol

Characteristic	Value in 0.1 N hydrochloric acid	Value in methanol
Absorption maxima (nm)	221[a], 281, 316	222, 283[a], 315
Beer's law limits (μg ml^{-1})[b]	1–10	10–100
Apparent molar absorptivity[b] (l mol^{-1} cm^{-1})	44731.7	6160.9

[a] Analytical wavelength for proposed method.
[b] At analytical wavelength.

tively. The optical characteristics for the solution of mefloquine hydrochloride in 0.1 N hydrochloric acid and in methanol are given in Table 3. Absorptivity scans over the UV wavelength range between 200 and 400 nm for a 4 μg ml^{-1} solution of mefloquine hydrochloride in 0.1 N hydrochloric acid and for a 40 μg ml^{-1} solution in methanol are shown in Figs. 1 and 2, respectively.

2.4. Stability

Stability of the solutions of mefloquine hydrochloride, used for preparing the calibration curves in both the methods, was ascertained by observing for changes in the absorbance at their respective analytical wavelengths over a period of 24 h.

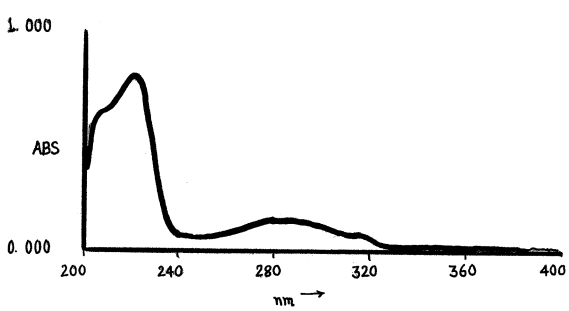

Fig. 1. Wavelength scan for mefloquine hydrochloride in 0.1 N hydrochloric acid.

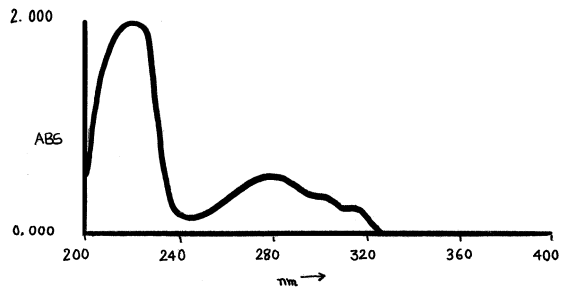

Fig. 2. Wavelength scan for mefloquine hydrochloride in methanol.

2.5. Selectivity

The selectivity of these methods for the estimation of the drug in presence of various tablet excipients such as starch, lactose, microcrystalline cellulose, sodium starch glycollate, talc and magnesium strearate was investigated.

A placebo comprising 63% w/w lactose, 15% w/w starch, 15% w/w microcrystalline cellulose, 4% w/w sodium starch glycollate, 2% w/w talc and 1% w/w magnesium stearate was prepared. A 1:1 blend of drug and placebo was prepared. Drug was then extracted from this blend using methanol and 0.1 N hydrochloric acid separately. The methanol and acid extracts were filtered through 589 Blue ribbon S & S filter paper circles of diameter 125 mm (Schliecher and Schuell, GmbH, Germany) and absorbance of the filtrates, appropriately diluted, was measured at 283 and 222 nm for the methanolic acid extracts, respectively. The above procedure was carried out in triplicate. Results of these determinations are included in Table 4.

2.6. Precision and accuracy

In order to determine precision and accuracy of the methods, solutions containing known amounts of pure drug were prepared and analyzed in three replicates. Drug was also extracted from a blend of placebo and known amount of drug and analyzed in three replicates. The analytical results obtained from these investigations for both methods are summarized in Table 4.

Table 4
Evaluation of accuracy and precision of the proposed methods

Exact amounts of drug added (mg)		Individual amounts found (mg); mean (S.D.)[a]		Coefficient of variation (CV)		Relative mean error (RME)		Confidence limits[b]	
ME_1	ME_2	ME_1	ME_2	ME_1	ME_2	ME_1	ME_2	ME_1	ME_2
(A) Pure drug									
20.10	20.17	18.24	19.94	6.66	1.62	0.0535	0.0135	18.93 ± 3.128	19.73 ± 0.796
20.02	20.24	20.38	19.90						
20.40	20.03	18.16	19.36						
		18.93 (1.26)	19.73 (0.32)						
25.17	25.06	25.73	25.02	10.54	1.317	0.0816	0.002	22.96 ± 6.008	25.05 ± 0.819
25.08	24.92	21.27	25.39						
25.22	25.10	21.88	24.73						
		22.96 (2.42)	25.05 (0.33)						
30.12	30.01	24.11	29.67	14.02	0.508	0.049	0.012	28.53 ± 9.931	29.53 ± 0.372
30.68	30.30	29.54	29.53						
30.40	29.94	31.93	29.38						
		28.53 (4.00)	29.53 (0.15)						
(B) Drug with placebo (1:1)									
10.05	11.00	9.63	11.67	6.74	4.53	0.031	0.109	10.31 ± 1.725	11.09 ± 1.246
10.11	10.60	10.29	10.81						
11.26	10.80	11.02	10.79						
		10.31 (0.695)	11.09 (0.502)						

[a] $n = 3$.
[b] Confidence limits at $P = 0.95$ and two degrees of freedom.

2.7. Comparison with pharmacopoeial method

Samples of pure drug were estimated using the method detailed in the European Pharmacopoeia [1]. Results of these estimations are tabulated in Table 5.

3. Results and discussion

Mefloquine hydrochloride in 0.1 N hydrochloric acid yields a characteristic curve when scanned in the ultraviolet wavelength range between 200 and 400 nm. The scan (Fig. 1) shows absorption maxima at 221, 281 and 316 nm (Table 3) in close proximity of the maxima reported (222, 283 and 317 nm) [21]. However, a reported peak at 303 nm was not found to be prominent in our experiment. The absorptivity at 222 nm was found to be 44731.7 l mol^{-1} cm^{-1}, which was in good agreement with the reported value [21] (41 800 l mol^{-1} cm^{-1}) and hence this wavelength was chosen as the analytical wavelength.

Mefloquine hydrochloride in methanol yields a characteristic curve similar to that obtained with 0.1 N hydrochloric acid with absorption maxima at 222, 283 and 315 nm (Table 3). Though the absorptivity at 222 nm was high it was not selected as analytical wavelength due to its proximity to the lower limit of the UV transparent region of methanol ($\approx$ 220 nm). The absorptivity at 283 nm was found more suitable and hence was selected for further investigations. The ultraviolet spectra in both the cases can be attributed mainly to the quinoline nucleus in the mefloquine hydrochloride molecule [23]. Correlation coefficients for ME_1 and ME_2 were found to be 0.999 and 0.997, respectively, signifying that a linear relation existed between absorbance and concentration of the drug.

Beer's law was found to be obeyed between 1 and 10 μg ml^{-1} for ME_1 and between 10 and 100 μg ml^{-1} for ME_2. Regression analysis was performed on the experimental data. The raw data along with the results of regression analysis (method of least squares) is shown in Tables 1 and 2 for ME_1 and ME_2, respectively. Regression equations for ME_1 and ME_2 were $y = 0.0982x + 0.02974$ and $y = 0.01377x + 0.02785$, respectively. The variance of the response variable, S^2_{yx}, for ME_1 was calculated to be 1.15×10^{-4} (six degrees of freedom) and for ME_2 was 1.745×10^{-4} (five degrees of freedom). These low values indicate the closeness of the experimental points to the least squares line. The fact is in concurrence with the low values of the standard error of the mean (S.E.M.) absorbances of the solutions used for preparing the calibration curve. The variances of both the methods were compared using 'F' distribution to determine whether they were significantly different from each other. The calculated 'F' value was found to be 1.52 whereas the tabulated 'F' value was 4.39 for six and five degrees of freedom in the denominator and numerator, respectively. There is no significant difference in the variances and hence no difference in variability exists between the two methods. The variances of the slope S^2_b, were calculated as 1.7×10^{-6} for ME_1 and 2.7×10^{-8} for ME_2. The higher slope of the regressed line of ME_1 (0.0982) as compared with that of ME_2 (0.01379) indicates higher sensitivity of ME_1 as compared with ME_2. This is supported by the narrower range in which Beer's law is obeyed for ME_1 (1–10 μg ml^{-1}) as com-

Table 5
Results of pharmacopoeial method for estimation of mefloquine hydrochloride

Exact amount of drug added (mg)	Individual amounts found (mg) mean (S.D.)[a]	Coefficient of variation (CV)	Relative mean error (RME)	Confidence limits[b]
0.3509	0.3478	2.02	0.0111	0.3461 ± 0.0174
0.3502	0.3525			
0.3514	0.3380			
	0.3461 (0.007)			

pared with that of ME_2 (10–100 μg ml^{-1}) and the higher absorptivity of mefloquine hydrochloride at 222 nm (44731.7 l mol^{-1} cm^{-1}) as compared with the same at 283 nm (6160.9 l mol^{-1} cm^{-1}). The value of Sandell's sensitivity coefficient for ME_1 (9.24×10^{-6} μg cm^{-2} per 0.001 abs unit) as compared with that for ME_2 (0.68×10^{-6} μg cm^{-2} per 0.001 abs unit) supports the above observation. The variances of the intercept, S_a^2, determined were 5.82×10^{-5} for ME_1 and 8.9×10^{-5} for ME_2. To examine whether these intercepts were significantly different from zero, the intercepts were subjected to a 't' test. The values of 't' were obtained as 3.89 for ME_1 (five degrees of freedom) and 2.95 for ME_2 (four degrees of freedom). The corresponding tabulated values of t were 4.03 and 4.60 at five and four degrees of freedom at the 1% level. Thus acceptance of the null hypothesis indicates that these intercepts were not significantly different from zero. Therefore, there are no interferences from the solvents used in the methods, i.e. 0.1 N hydrochloric acid and methanol.

The stability of mefloquine hydrochloride in 0.1 N hydrochloric acid and in methanol was monitored over a period of 24 h. ANOVA studies of the mean absorbance values of the solutions of different concentrations at preselected time intervals indicated that no significant difference existed between the readings. Thus mefloquine hydrochloride is stable over a period of 24 h in both, dilute acid and methanol.

Estimation of mefloquine hydrochloride was carried out, using both the methods, in the presence of various commonly used tablet excipients at the levels they are normally used. From Table 4, it can be seen that there is no significant difference between the amount added to the placebo and the amount recovered (computed 't' for $ME_1 = 0.772$, for $ME_2 = 3.76$, calculated $t = 4.30$ at two degrees of freedom) $P \leq 0.05$. Thus, excipients like starch, lactose, microcrystalline cellulose, sodium starch glycollate, talc and magnesium stearate did not interfere with the estimation. Also, the filtration medium did not absorb the drug to any extent.

In order to determine the precision and accuracy of the methods, known amounts of pure drug were subjected to recovery studies, using both the methods, in triplicate. Table 4 summarizes the results of these investigations. Accuracy of each method was ascertained by using the 't' test at each level. The computed 't' values at 20, 25 and 30 mg for ME_1 are 1.471, 1.450 and 0.637, respectively, and for ME_2 are 1.461, 0.262 and 4.04, respectively. These values are lower than the tabulated 't' value of 4.30 ($P \leq 0.05$) indicating no significant difference between the added and the estimated quantity. To compare the accuracy of both methods the relative mean error (RME) of each method was calculated and is shown in Table 4. The relative mean error for ME_2 is less than that for ME_1 at each level signifying that ME_2 is more accurate as compared with ME_1.

The precision of both the methods was evaluated using the S.D. of the results, the coefficient of variation and the F-test. The computed F-values at 20, 25 and 30 mg were 15.5, 53.78 and 711.1, respectively, which were found to be significant at $P \leq 0.1$, $P \leq 0.05$ and $P \leq 0.01$, respectively, (tabulated F-values = 9.00, 19.0 and 99.0, respectively). This indicates that a significant difference exists between the precision of both the methods. The higher S.D. of the results and the higher coefficient of variation associated with ME_1 as compared with those of ME_2 make the former a relatively less precise method. This is also reflected in the larger confidence limits (Table 4) for different levels when ME_2 is used for estimation. The decreasing level of significance with increasing amounts of drug points to the importance of amount of drug when using method ME_1 probably due to the limited solubility of mefloquine hydrochloride in dilute acid [23]. The results of recovery of drug from the drug–placebo blend (Table 4) supports the above observations.

The proposed methods were compared with the method given in the European Pharmacopoeia [1]. The pharmacopoeia method involved non-aqueous titration using perchloric acid. Results of the determination in triplicate are given in Table 5. From the results, it can be seen that the proposed methods are comparable to the pharmacopoeial method in terms of accuracy and precision.

4. Conclusions

Mefloquine hydrochloride can be estimated using both the methods ME_1 (0.1 N hydrochloric acid) and ME_2 (methanol) at 222 and 283 nm, respectively. Both methods have the advantages of simplicity, stability, sensitivity, reproducibility and accuracy. However, method ME_1 is associated with higher sensitivity but lower precision as compared with ME_2. The non-interference of tablet excipients makes the methods suitable for the estimation of the drug in tablets and hence can be used for routine quality control of mefloquine hydrochloride formulations. It is recommended that ME_1 be used when the amount of mefloquine hydrochloride in the formulation is low since mefloquine hydrochloride has limited solubility in dilute acid. Mefloquine hydrochloride, being freely soluble in methanol, ME_2 can be used for formulations containing higher amounts of mefloquine hydrochloride. It is also recommended strongly that a bath sonicator be used when ME_1 is being used for estimation to ensure fast and complete dissolution of the drug.

Results of the above study indicates the suitability of the methods to estimate mefloquine hydrochloride in bulk as well as in formulations. The developed methods are comparable to the official method elaborated in the European Pharmacopoeia. They may also be selected as an alternative to the existing, time-consuming and expensive methods like gas chromatography and high performance liquid chromatography (HPLC).

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References

[1] European Pharmacopoeia, third ed., Supplement, Council of Europe, Strasbourg, 2001, pp. S1109–S1111.
[2] J.M. Grindel, P.F. Tilton, R.S. Shaffer, J. Pharm. Sci. 66 (1977) 834–837.
[3] I.M. Kapetanovic, J.D. Di Giovanni, J. Bartosevich, V. Melendez, J. Van Bredow, M. Heiffer, J. Chromatogr. Biomed. Appl. 28 (1983) 209–215.
[4] P.J. Arnold, O.V. Stetton, J. Chromatogr. 353 (1986) 193–200.
[5] Y. Bergqvist, S. Eckerbom, N. Larsson, F.C. Churchill, J. Chromatogr. Biomed. Appl. 71 (1988) 295–305.
[6] Y. Bergqvist, U. Hellgren, F.C. Churchill, J. Chromatogr. Biomed. Appl. 76 (1988) 253–263.
[7] Y. Bergqvist, S. Eckerbom, H. Larsson, M. Malek Zadeh, J. Chromatogr. Biomed. Appl. 109 (1991) 169–177.
[8] M.D. Edstein, J.D. Lika, T. Chongsuphajaisiddhi, A. Sabchareon, A.K. Webster, Ther. Drug Monit. 13 (1991) 146–151.
[9] Y. Bergqvist, M. Doverskog, J. Al-kabbani, J. Chromatogr. B Biomed. Appl. 652 (1994) 73–81.
[10] Y. Bergqvist, J. Al-kabbani, C. Petterson, N.H. Huynh, J. Chromatogr. Biomed. Appl. 131 (1993) 217–224.
[11] L. Wallen, O. Ericsson, I. Wikstrrom, U. Hellgren, J. Chromatogr. B Biomed. Appl. 655 (1994) 153–157.
[12] Y.H. Qiu, S. Kitamura, J.K. Guillory, Pharm. Res. 9 (1992) 1640–1643.
[13] T. Nakagawa, T. Higuchi, J.L. Hasiam, R.D. Shaffer, D.W. Mendenhall, J. Pharm. Sci. 68 (1979) 718–721.
[14] D.E. Schwartz, U.B. Ranalder, Biomed. Mass Spectrom. 8 (1981) 589–592.
[15] P. Heizmann, R. Geschke, J. Chromatogr. Biomed. Appl. 36 (1984) 411–417.
[16] D. Dadgar, J. Climax, R. Lambe, A. Darragh, J. Chromatogr. Biomed. Appl. 38 (1985) 47–54.
[17] J.M. Neal, W.N. Howald, K.L. Kunze, J. Chromatogr. B Biomed. Appl. 661 (1994) 265–269.
[18] B. Betschart, A. Sublet, S. Steiger, J. Planar Chromatogr. Mod. TLC 4 (1991) 111–114.
[19] J. Fidanza, J.J. Aaron, Anal. Chim. Acta 227 (1989) 325–330.
[20] S. Fanali, E. Camera, J. Chromatogr. A 745 (1996) 17–23.
[21] P. Lim, in: K. Florey (Ed.), Analytical Profiles of Drug Substances, vol. 14, Academic Press, Florida, 1985, pp. 157–180.
[22] The Indian Pharmacopoeia, third ed., Controller of Publications, New Delhi, 1985, pp. A190.
[23] I.L. Finar, in: I.L. Finar (Ed.), Organic Chemistry, vol. II, fifth ed., Longman Scientific and Technical, Singapore, 1988, pp. 17–19.