Application of Oxidative Coupling Reactions for the Estimation of Monobenzone in Bulk Sample and Dosage Forms

G.P.V. MALLIKARJUNA RAO†, P. ARUNA DEVI†, K.M.M. KRISHNA PRASAD†
and C.S.P. SASTRY*
*Department of Organic Chemistry, Foods, Drugs & Water, School of Chemistry
Andhra University, Visakhapatnam-530 003, India*

Five simple and sensitive visible spectrophotometric methods (method A, method B, method C, method D and method E) have been developed for the estimation of monobenzone (MB) in bulk and pharmaceutical formulations. These methods are based on the oxidative coupling reactions of monobenzone with 3-methyl-2-benzothiazolinone hydrazone (MBTH) in presence of sodium metaperiodate ($NaIO_4$) (method A, λ_{max} 520 nm), 4-aminophenazone (4-AP) in presence of potassium ferricyanide ($K_3[Fe(CN)_6]$ (method B, λ_{max} 500 nm), N,N-dimethyl amino-*p*-phenylenediamine (DMPD) in presence of sodium metaperiodate ($NaIO_4$) (method C, λ_{max} 640 nm), *p*-amino-phenol (PAP) in presence of molecular oxygen (method D, λ_{max} 620 nm) and 2,6-dichloroquinone-4-chlorimide (DCQC) in presence of buffer pH 9.4 (method E, λ_{max} 600 nm). Beer's law limits, precision and accuracy of these methods are checked by the UV reference method. The results obtained in the five methods are reproducible and are statistically validated and found to be suitable for the assay of monobenzone in bulk and pharmaceutical formulations.

Key Words: Oxidative coupling reactions, Estimation, Monobenzone.

INTRODUCTION

Monobenzone[1-3] (MB) [phenol, 4(phenyl methoxy)] is a demelanizer. It acts by interfering with the formation of melanin, which is the principal cutaneous pigment. The reported analytical procedures for the estimation of MB in bulk samples and in unit dosage forms include HPLC[4-7], TLC[8] and electrophoresis[9] techniques. During the course of our efforts to develop simple, sensitive and analytical procedures for various drugs, five analytical methods based on oxidative coupling reactions of MB are developed and presented in this paper. In these methods, the reagents are 3-methyl-2-benzothiazolinone hydrazone

(MBTH), sodium metaperiodate (NaIO$_4$) (method A), 4-aminophenazone (4-AP), potassium ferricyanide K$_3$[Fe(CN)$_6$] (method B), N,N-dimethyl amino-*p*-phenylene diamine (DMPD), sodium metaperiodate (NaIO$_4$) (method C), *p*-aminophenol (PAP) (method D) and 2,6-dichloroquinone-4-chlorimide (DCQC) (method E). All these methods are applicable to the determination of MB in bulk forms and pharmaceutical formulations.

A double-beam Shimadzu (UV-140) spectrophotometer with 1 cm matched quartz cells and a Systronics 106 visible spectrophotometer, with 1 cm matched glass cells, were used for the absorbance measurements in the reference and proposed methods respectively. An Elico LI-120 digital pH meter was used for pH measurements.

All reagents and chemicals used were of analytical grade and all solutions were prepared with double distilled water. Freshly prepared solutions were always used.

Aqueous solutions of 8.55×10^{-3} M MBTH (Fluka) and 9.35×10^{-3} M NaIO$_4$ (BDH) were prepared for method A. Aqueous solutions of 2.46×10^{-2} M 4-Ap (Ferack), 6.07×10^{-2} M K$_3$[Fe(CN)$_6$] (BDH) and 9.43×10^{-2} M Na$_2$CO$_3$ (Ranbaxy) were prepared for method B. Aqueous solutions of 1.19×10^{-3} M DMPD (Merck) and 4.67×10^{-3} M NaIO$_4$ (BDH) were prepared for method C. Aqueous solutions of 3.66×10^{-3} M PAP (Loba) and 5.0×10^{-1} M Na$_2$CO$_3$ were prepared for method D. Aqueous solutions of 1.9×10^{-3} M DCQC (Loba) and pH 9.4 borate buffer were prepared for method E.

Standard drug solution

A 200 μg mL^{-1} solution of MB was prepared by dissolving 20 mg of it in 5 mL of 0.1 M NaOH, followed by dilution to 100 mL with distilled water and this stock solution was diluted stepwise with distilled water to obtain the working standard solutions of 40 μg mL^{-1} for method E, 80 μg mL^{-1} for methods C and D, 100 μg mL^{-1} for methods A and B respectively.

Sample solution

The commercially available formulations for MB are in the form of cream. A quantity of cream equivalent to 100 mg of MB was treated with 3×15 mL portions of warm chloroform and the chloroform extract was transferred into a separatory funnel. Then it was extracted with 0.05 M NaOH (3×10 mL) and finally with 2×10 mL portions distilled water. The combined aqueous layers were taken in 100 mL volumetric flask and then diluted to 100 mL with distilled water to obtain 1 mg mL^{-1} solution. This 1 mg mL^{-1} (stock sample solution) was further diluted appropriately with distilled water to get working sample solutions of 40 μg mL^{-1} for method E, 80 μg mL^{-1} for methods C and D, 100 μg mL^{-1} for methods A and B respectively.

Recommended Procedures

Method A: Aliquots of (0.5–2.5 mL; 100 μg mL^{-1}) of MB solution and 1.0 mL each of 8.55×10^{-3} M MBTH solution and 9.35×10^{-3} M NaIO$_4$ solution were delivered into a series of 25 mL calibrated tubes and kept aside for 5 min. 3 mL of acetone was added to each tube and diluted to 25 mL with distilled water. The absorbances were measured within 45 min at 520 nm against a reagent blank

prepared in a similar manner omitting the drug. The coloured species was stable for 1 h. The drug concentration was read out from a calibration curve prepared under identical conditions.

Method B: Aliquots of MB (0.5–3.0 mL; 100 µg mL^{-1}) solution, 0.6 mL (9.43 × 10^{-2} M) sodium carbonate, 1.0 mL of (2.46 × 10^{-2} M) 4-AP and 1.0 mL of (6.07 × 10^{-2} M) K$_3$[Fe(CN)$_6$] were added successively into a series of 25 mL calibrated tubes and the total volume in each flask was brought to 9.0 mL with distilled water and kept aside for 5 min. Then diluted to the mark with distilled water and the absorbances were measured at 500 nm against reagent blank. The colored species was stable for 2 h. The drug concentration was deduced from a calibration curve.

Method C: Aliquots of MB (0.5–2.5 mL; 80 µg mL^{-1}) were delivered into a series of 25 mL graduated test tubes. One mL each of (1.19 × 10^{-3} M) DMPD solution and (4.67 × 10^{-3} M) NaIO$_4$ solutions were added successively and kept aside for 30 min at room temperature. The volume was brought up to the mark with distilled water and the absorbances were measured at 640 nm against a reagent blank prepared in a similar manner. The coloured species was stable for 1 h. The amount of MB was calculated from the calibration curve.

Method D: To aliquots of MB (1.0–5.0 mL; 80 µg mL^{-1}) solution, 2.0 mL of (3.66 × 10^{-3} M) PAP solution, 3.0 mL of (5.0 × 10^{-1} M) sodium carbonate solution, 3.0 mL of isopropanol were added successively into a series of 25 mL calibrated tubes and kept aside for 45 min at room temperature and made upto 25 mL with distilled water. The absorbance was measured at 620 nm against a reagent blank within 1 h. The drug concentration was calculated from a calibration graph prepared with a standard solution under identified conditions.

Method E: Aliquots of MB (1.0–6 .0 mL, 40 µg mL^{-1}) were delivered into a series of 25.0 mL calibrated test tubes. Then 5.0 mL of buffer (pH 9.4, borate buffer) and 2.0 mL of (1.9 × 10^{-3} M) DCQC were added successively and the volume was made up to 10.0 mL with distilled water. After 10 min, the volume was made up to 25 mL with distilled water. The absorbances of the coloured species were measured at 600 nm against a reagent blank prepared simultaneously. The coloured species was stable for 45 min. The amount of drug (MB) was computed from the appropriate calibration curve.

The optical characteristics such as absorbance maxima, Beer's law limits, molar absorptivity, Sandell's sensitivity, regression equation and correlation coefficient obtained by least squares treatment of these results are given in Table-1. The precision of each method was tested by analysing the six replicate samples (3/4 amount of upper Beer's law limit of MB are also given in Table-1). The per cent relative standard deviation and the per cent range of error at 95% confidence level of each method are given in Table-1.

Commercial formulations (cream) containing MB were successfully analysed by the proposed methods. The results obtained by the proposed and UV reference methods for dosage forms were compared statistically by means of the F- and t-tests and were found not to differ significantly[10] at the 95% confidence level. As an additional check of accuracy of the proposed methods, recovery experiments were performed by adding a fixed amount of the drug to the preanalysed formulations and the results are presented in Table-2. These results indicate that the commonly

used excipients and additives in the creams such as ointment bases—oleaginous bases (vegetable oil, liquid paraffin, hydrocarbon like petroleum), absorption bases (e.g., hydrophilic petrolatum, anhydrous lanolin), water soluble bases (mixture of high and low molecular weight polyethylene glycols etc.) and water removable bases (vanishing cream) and other additives like preservatives, antioxidants, chelating agents or perfumes did not interfere with determination of MB by the five proposed methods.

TABLE-1

OPTICAL CHARACTERISTICS AND PRECISION OF THE PROPOSED METHODS FOR MONOBENZONE

Parameter	Method A	Method B	Method C	Method D	Method E
λ_{max} (nm)	520	500	640	620	600
Beer's law limits ($\mu g\ mL^{-1}$)	0.8–10	1–12	0.64–8.0	1.2–16	0.72–9.6
Molar absorptivity (L mole^{-1} cm^{-1})	1.401×10^4	1.016×10^4	1.556×10^4	7.945×10^3	1.382×10^4
Sandell's sensitivity ($\mu g\ cm^{-2}$/ 0.001 absorbance unit)	0.014	0.019	0.012	0.025	0.014
Regression equation Y*					
Slope (b)	6.99×10^{-2}	5.03×10^{-2}	7.75×10^{-1}	3.91×10^{-2}	6.88×10^{-2}
Intercept (a)	2.0×10^{-3}	1.06×10^{-3}	1.0×10^{-4}	3.06×10^{-3}	8.66×10^{-4}
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999	0.9999
relative standard deviation (%)†	0.239	0.477	0.470	0.185	0.389
% range of error (confidence limits)					
0.05 level	0.321	0.501	0.494	0.195	0.409
0.01 level	0.413	0.785	0.775	0.305	0.641

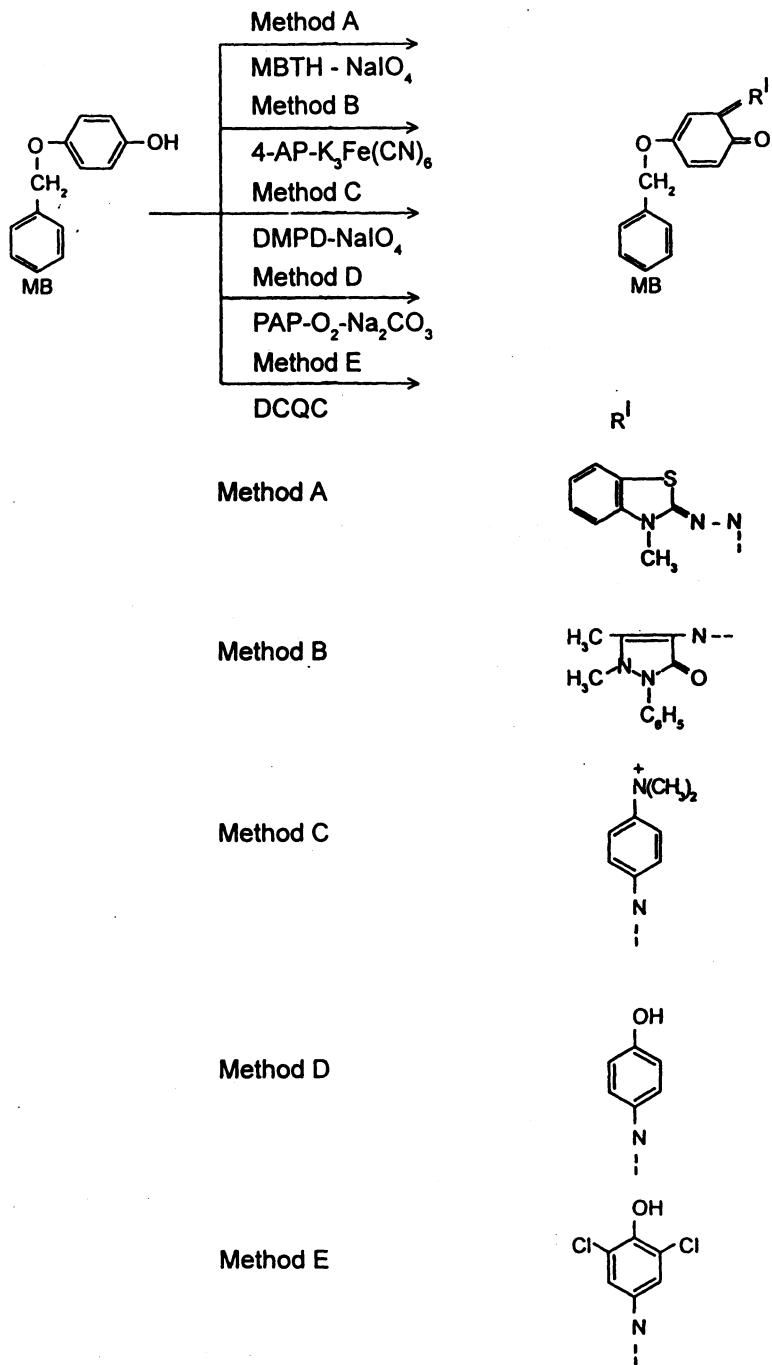*y = a + bc where c is concentration in mg mL^{-1} and Y is the absorbance unit.

† Six replicate samples.

Chemistry of coloured species

Method A: MBTH is a well known analytical reagent for the determination of phenolic compounds[11, 12]. Under the reaction conditions MBTH undergoes oxidation losing two electrons and one proton and forms an electrophilic intermediate, which has been postulated to be the active coupling species. Electrophilic attack by this intermediate at the most nucleophilic site of MB that is available, i.e., the position *ortho* to the phenolic hydroxyl group (as *para* position is blocked), results in the formation of the coupled product which is spontaneously oxidised by NaIO$_4$ to form the coloured species as shown in Scheme-1.

Method B: 4-AP in presence of alkaline oxidising agent yields a quinone imine which in turn is known to spontaneously react with compounds to yield a red colored antipyrine dye[13, 14]. The phenolic hydroxyl groups present in MB render it an extremely suitable substrate for this reaction. The coloured species resulting from such reaction may be formulated in Scheme-1.

Method C: DMPD undergoes oxidation in the presence of NaIO$_4$ to the highly p-N, N-dimethyl benzoquinone diimine (PDBQDI)[15, 16]. The substrate MB then

Method A

MBTH - NaIO$_4$

Method B

4-AP-K$_3$Fe(CN)$_6$

Method C

DMPD-NaIO$_4$

Method D

PAP-O$_2$-Na$_2$CO$_3$

Method E

DCQC

MB

MB

R^I

Method A

Method B

Method C

Method D

Method E

Scheme-1   Structure of monobenzone (MB) and its reaction products with MBTH, 4-AP,
          DMPD or PAP in presence of oxidant and DCQC

TABLE-2

ANALYSIS OF MONOBENZONE (MB) IN PHARMACEUTICAL FORMULATIONS

Formulations (cream)[a]	Labelled amount (mg)	Amount found by proposed methods[a], mg					Reference method (mg)[b]	% Recovery by proposed methods[c]				
		Method A	Method B	Method C	Method D	Method E		Method A	Method B	Method C	Method D	Method E
I	100	99.69±0.516	99.55±0.508	99.77±0.21	99.77±0.21	99.74±0.23	99.93±0.144	99.69±0.516	99.55±0.50	99.77±0.21	99.77±0.20	99.70±0.23
		$F=1.326$	$F=1.28$	$F=4.9$	$F=4.55$	$F=3.79$						
		$t=1.204$	$t=0.810$	$t=0.70$	$t=0.70$	$t=0.2$						
II	100	100.17±0.464	99.99±0.70	99.64±0.41	99.67±0.41	100.19±0.62	100.23±0.73	100.17±0.464	99.99±0.70	99.64±0.41	99.67±0.41	100.19±0.62
		$F=2.47$	$F=1.08$	$F=3.17$	$F=3.17$	$F=1.38$						
		$t=0.379$	$t=0.282$	$t=0.90$	$t=1.4$	$t=0.58$						
III	100	100.07±0.132	100.08±0.79	99.86±0.25	99.73±0.45	99.77±0.25	99.55±0.556	100.07±0.132	100.08±0.79	99.86±0.25	99.73±0.45	99.77±0.25
		$F=3.9$	$F=2.01$	$F=4.96$	$F=1.52$	$F=4.94$						
		$t=1.0$	$t=0.68$	$t=2.39$	$t=1.97$	$t=0.466$						
IV	100	99.61±0.40	100.33±0.57	99.66±0.42	99.86±0.23	99.54±0.45	99.7±0.274	99.61±0.40	100.33+0.57	99.66±0.42	99.86±0.23	99.54 ±0.45
		$F±2.13$	$F=2.11$	$F=2.41$	$F=1.37$	$F=2.77$						
		$t=0.24$	$t=1.03$	$t=1.0$	$t=1.90$	$t=0.393$						

[a] Average + standard deviation of six determinations; the t- and F-values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limts: $t=2.57$, $F=5.05$.

[b] U.V. reference method developed in laboratory for MB in 0.1 M NaOH (λ_{max} 305 nm).

[c] After adding three different amounts of pure MB to the preanalysed pharmaceutical formulations.

couples with this species as shown in Scheme-1 to yield a leuco dye that is spontaneously oxidised to the indo dye.

Method D: PAP undergoes oxidation in alkaline medium to give PBQMI[17], which is in the highly reactive form couples with MB to give indo phenol as shown in Scheme-1. Above pH 9.0 the dyes exist in solution as blue anionic form.

Method E: Phenols undergo oxidative coupling reaction with DCQC to give indo phenols[18-20] in alkaline medium. The drug undergoes coupling reaction with DCQC to give colored species as shown in Scheme-1.

Conclusion

There is no report for the assay of monobenzone using visible spectrophotometry. The five proposed methods (A–E) exploit the structural features (phenolic hydroxyl) of MB molecule. The higher λ_{max} values of all the proposed methods have a decessive advantage since the interferences from the associated ingredients should be generally far less at higher wavelengths than at lower wavelengths. The sensitivity order of proposed methods is method C > method A > method E > method B > method D and the λ_{max} order of the coloured species is method C > method D > method E > method A > method B. Thus the proposed visible spectrophotometric methods are simple and sensitive with reasonable precision and accuracy and constitute the routine determination of MB in bulk form and unit dosage forms and provide a wide choice, depending upon the availability of chemicals, needs of a specific situation and nature of the ingredients present in the sample under analysis.

REFERENCES

1.   Martha Windholz (Ed.), Merck Index, 12th Edn., Merck Sharp and Dhome Research Labs, U.S.A., p. 6331 (1996).
2.   Martindale, The Extra Pharmacopeia, 32nd Edn., The Pharmaceutical Press, London, p. 1088.3 (1999).
3.   Remington's The Science and Practice of Pharmacy, 19th Edn., p. 883 (1995).
4.   Otto and Heisz, *Labor Praxis*, **2**, 28 (1978).
5.   L. Gagliardi, A. Amato, G. Cavazzuti, F. Chementi, A. Bolasco and D. Tonelli, *J. Chromatogr.*, **404**, 267 (1987).
6.   M. Herpor-Borremanas and M.O. Masse, *Int. J. Cosmet. Sci.*, **8**, 203 (1986).
7.   M. Borremans, J. De Beer and L. Goeyens, *Chromatographia*, **50**, 346 (1999).
8.   F.J. Van de Vaart and A. Hulshoff, *Pharm. Week Bl., Sci. Ed.*, **5**, 113 (1983).
9.   C. Desiderio, L. Ossicini and S. Fanali, *J. Chromatographia.* **887**, 489 (2000).
10. D.L. Massart, B.G.M. Vandegingtc, S.N. Deming, Y. Michotte and L. Kaufinan, Chemometrics: a Text Book, Elsevier, Amsterdam, p. 293 (1988).
11. E. Kommas and E. Michael, *Arch. Pharm. Chem. Sci. Ed.*, **10**, 146 (1982).
12. C.S.P. Sastry and B.S. Sastry, *East Pharm.*, **29**, 31 (1986).
13. E. Emerson, H.H. Beachem and L.C. Beagle, *J. Org. Chem.*, **8**, 417 (1943).
14. C.S.P. Sastry, T.E. Diwakar and U.V. Prasad, *East Pharm.*, **28**, 135 (1985).
15. B. Camber, *Nature*, **175**, 1085 (1955).
16. A.M. Taha and G.S. Gomaa, *Am. Cosmet. Prefumery*, **87**, 41 (1971).
17. J.F. Cosbelt, *J. Chem. Soc. (B).*, 823 (1969).
18. H.D. Gibbs, *Chem. Rev.*, **3**, 291 (1927).
19. ———— , *J. Biol. Chem.* **72**, 649 (1927).
20. E. Boyland, D. Manson, J.B. Solomon and G.H. Wiltshire, *Biochem. J.*, **53**, 420 (1953).