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Determination of meloxicam in bulk and pharmaceutical formulations

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Abstract

Three sensitive and reproducible methods for quantitative determination of meloxicam (mel) in pure form and in pharmaceutical formulations are presented. The first method is high performance liquid chromatography by which the drug is determined in the presence of its degradation products over concentration range 100–500 μg ml^{-1} with mean percentage accuracy 100.13 ± 0.53. The second method is based on measuring the absorbance of the formed neutral complex between basic methylene blue and mel in phosphate buffer (pH 8) at $\lambda = 653.5$ nm over concentration range 1–5 μg ml^{-1} with mean percentage accuracy 99.12 ± 1.18. The third method is based on reaction between 2,3-dichloro-5,6-dicyano-*p*-benzoquinone resulting in the formation of an intense orange red coloured product after heating in a boiling water bath for 5 min. The coloured product exhibits an absorption maximum at 455 nm, over concentration range 40–160 μg ml^{-1} with mean percentage accuracy 100.53 ± 1.04.

Keywords: Meloxicam; HPLC; Stability-indicating method; DDQ; Methylene blue

1. Introduction

Meloxicam 4-hydroxy-2-methyl-*N*(5-methyl-2-thiazolyl)-2-H-1,2-benzothiazine-3-carboxamide-1,1-dioxide is a non steroidal anti-inflammatory drug [1,2]. Few methods have been reported for the determination of meloxicam (mel) including non aqueous titration [3], spectrophotometric [4–7], high performance liquid chromatography (HPLC) methods [8–12]and densitometric method [13]. The proposed HPLC method has the advantage of being selective and capable of quantitative determination of mel in the presence of its degradation products which may result from the hydrolysis of mel under inappropriate storage conditions of humidity and high temperature. The proposed HPLC method had a percentage recovery of 99.25–100.67, %RSD is 0.53 with intra- and inter-day assay variations of 0.36–0.87 and 0.39–0.59%, respectively, while the reported HPLC method [8] shows percentage recovery of

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97–103, with intra- and inter-day assay variations of 3.1–5.7 and 5.2–6.3%, respectively. The accuracy of the proposed HPLC method is better than the reported method [12] as the percentage recovery of the latter method is 97.7–101.9 and %RSD is 1.91–5.79. The proposed colourimetric methods have the advantages of simplicity, availability of equipment and low cost. In addition they are more accurate and more sensitive than the published spectrophotometric methods as methylene blue (MB) method was capable of determining up to 1 μg ml^{-1} of mel with percentage recovery of 98.14–101.00 and %RSD 1.18, while the proposed 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) method can determine mel up to 40 μg ml^{-1} with a percentage recovery of 99.50–101.80 and %RSD is 1.03. The reported spectrophotometric method [4] showed a percentage recovery of 98.9–102.80 and %RSD is 1.6–2.9. The reported spectrophotometric method [6] was applied to tablets only while all the proposed methods were to both tablets and suppositories. The official method [3] can determine mel up to 50 mg. All the proposed methods were applied for the assay of pharmaceutical formulations without the interference of excepients. Statistical interpretation of the obtained results was made.

2. Experimental

2.1. Material

1) Mel standard was kindly supplied by Boehringer Ingelheim. The purity of the sample was found to be 99.51 ± 0.59 according to the official method [3].
2) Mobic tablets (Bohringer Ingelheim Gmbh, Germany), batch number: 606814 and 50001, labelled to contain 7.5 and 15 mg mel/tablet, respectively.
3) Mobitil tablets (Medical Union Pharmaceuticals Abu-Sultan Ismailia Egypt), batch number: 001252 and 001143 labelled to contain 7.5 and 15 mg mel/tablet, respectively.
4) Mobic suppository (Bohringer Ingelheim Gmbh), batch number: 44423 labelled to contain 15 mg/suppository.
5) Mobitil suppository (Medical Union Pharmaceuticals Abu-Sultan Ismailia Egypt), batch number: 001122 labelled to contain 15 mg / suppository.
6) Mexicam suppository (Delta pharm, Cairo, Egypt), batch number: 67662, labelled to contain 15 mg/suppository.

2.2. Reagents and apparatus

1) HPLC Hewlett Packard series 1100 equipped with a quaternary pump, Diode array detector and a manual injector 20 μl loop.
2) Spherisorb ODS (200 × 4.6 mm i.d.) particle size of 5 μm.
3) Ultraviolet/visible spectrophotometer Shimadzu 1601 pc.
4) Ultrasonic, J.P. Selecta, s-a, CD. 300513, Espain.
5) Hettich centrifuge.
6) Mobile phase: methanol: acetate buffer pH 4.3 (45:55, v/v). All reagents are HPLC grade from lab scan analytical sciences. The buffer is prepared by adjusting the pH of 0.4 M sodium acetate with glacial acetic acid to pH 4.3.
7) Phosphate buffer pH 8 [14]
8) MB (Aldrich Chemical Co. Ltd. Gillingham, Dorset-England). A solution of 0.1% w/v in phosphate buffer pH 8 was prepared.
9) Chloroform (Acros Organics, New Jersey, USA)
10) DDQ (Acros organics) 0.1% w/v solution in acetonitrile.
11) Acetonitrile (Lab scan analytical sciences). A solution of 0.1% was prepared in acetonitrile.

2.3. Preparation of degradation products

Two degradation products were separated from alkaline hydrolysis of mel namely: 5-methyl-2-aminothiazole and benzathine carboxylic acid. They were prepared according to the method of Bebawy [13].

2.4. Stock solutions

2.4.1. Stock solution A

Weigh accurately about 50 mg mel into a 50-ml volumetric flask, add 0.5 ml 1 N sodium hydroxide, 30 ml methanol (HPLC grade), shake well to dissolve the powder and complete to volume with the same solvent.

2.4.2. Stock solution B

Weigh accurately about 25 mg mel into a 100-ml volumetric flask, add 5 ml methanol, 60 ml phosphate buffer, sonicate for 15 min. complete to volume with buffer, further dilute to obtain a solution of 0.1 mg ml^{-1}.

2.4.3. Stock solution C

Prepare a solution of 0.5 mg ml^{-1} mel in acetonitrile.

2.4.4. Stock solution D

Prepare a solution of 1 mg ml^{-1} of the degradation products in methanol (HPLC grade) containing 0.5 ml 1 N sodium hydroxide.

2.5. Pharmaceutical preparations

2.5.1. Tablets test solutions (At, Bt, Ct) 7.5, 15 mg mel/tab

Grind 20 tablets from each concentration (for each pharmaceutical preparation separately; mobic and mobitil). Prepare each of the following test solutions.

2.5.1.1. Test solution At. To the powder equivalent to 50 mg mel add 0.5 ml 1 N sodium hydroxide in 50-ml volumetric flask, add 30 ml methanol (HPLC grade), sonicate for 45 min. Complete to volume with the same solvent, filter.

2.5.1.2. Test solution Bt. To the powder equivalent to 25 mg mel add 5 ml methanol in 100-ml volumetric flask, 60 ml phosphate buffer, sonicate for 45 min. Complete to volume with buffer, filter, further dilute to obtain solution of concentration 0.1 mg ml^{-1} in buffer.

2.5.1.3. Test solution Ct. To the powder equivalent to 25 mg mel add 30 ml acetonitrile in 50-ml volumetric flask, sonicate for 45 min. Complete to volume with the same solvent, centrifuge for 15 min (3000 rpm)and separate the supernatant.

2.5.2. Suppository test solutions (As, Bs, Cs) 15 mg mel/suppository

Melt 20 suppositories of each pharmaceutical preparation (mobic, mobitil, mexicam) separately. Accurately weigh an amount of the melt to prepare test solutions similar to test solutions At, Bt, Ct, namely test solutions As, Bs and Cs, respectively (cool before filtration).

2.6. Procedures

2.6.1. HPLC method

2.6.1.1. Construction of calibration curve. Transfer accurately measured aliquots of stock solution A to prepare solutions of 100–500 μg ml^{-1} in methanol (HPLC grade), inject 20 μl of each solution to HPLC (Hewlett packard 1100) using mobile phase methanol: acetate buffer pH 4.3 (45:55,v/v). Carry out detection at 365 nm, flow rate 1 ml min^{-1}. Construct calibration curve.

2.6.1.2. Assay of prepared mixtures. Transfer accurately measured aliquots from stock solution A equivalent to 1–5 mg mel in 10-ml volumetric flask, add from 10 to 90% of degradation products using stock solution D. Complete to volume with methanol, proceed as under calibration curve starting from ' inject 20 μl . . .'. Detection is carried out at 365 nm (degradation products are detected at 254 nm). Calculate concentration of mel from regression equation.

2.6.1.3. Assay of tablets and suppositories. Proceed as under construction of calibration curve by using different aliquots of test solution At and As equivalent to 1–5 mg mel. Calculate concentration from regression equation.

2.6.2. Methylene blue complexation method

2.6.2.1. Construction of calibration curve. Aliquots of standard stock solution B equivalent to 0.05–0.25 mg were transferred in a series of 125 ml separating funnels. Adjust the volume to 10 ml with phosphate buffer, add 5 ml 0.1% w/v MB and mix shake with 25, 10, 10 ml portions of chloroform. Filter the chloroformic extracts over anhydrous sodium sulfate (~ 0.5 g) and collect in 50-ml volumetric flask. Complete to volume with chloroform, measure the absorbance at 653.5 nm against reagent blank within 30 min. Construct calibration curve.

2.6.2.2. Assay of tablets and suppositories. Proceed as under construction of calibration curve using different aliquots of test solution Bt and Bs equivalent to 0.05–0.25 mg mel. Calculate concentration from regression equation.

2.6.3. DDQ method

2.6.3.1. Construction of calibration curve. Transfer accurately measured aliquots equivalent to 0.4–1.6 mg mel to a series of test tubes add 3 ml of 0.1% w/v DDQ in acetonitrile. Heat in a boiling water bath for 5 min, cool, transfer to a series of 10-ml volumetric flasks. Complete to volume with acetonitrile, measure the absorbance at 455 nm against reagent blank. Construct calibration curve.

2.6.3.2. Assay of tablet and suppositories. Proceed as under calibration curve using different aliquotes of test solution Ct and Cs equivalent to 0.4–1.6 mg mel. Calculate concentration from regression equation.

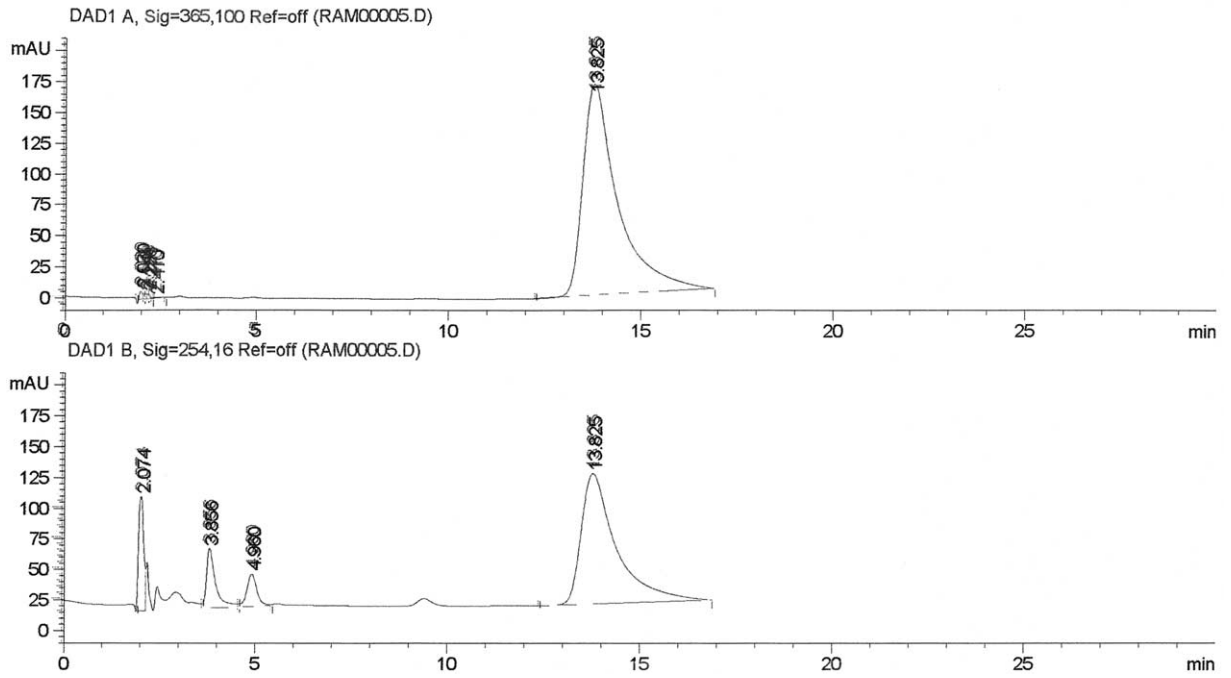

Fig. 1. A typical HPLC chromatogram of mel and its degradation products Rt of mel is 13.83 min, Rt of benzothiazine is 4.96 min and Rt of 5-methyl-2-aminothiazole is 3.86 min.

3. Results and discussion

3.1. HPLC method

3.1.1. Optimisation of chromatographic procedure

Several mobile phases were tried, those that were known to quantify mel showed bad resolution from its degradation products. The chosen mobile phase showed good resolution Fig. 1.

Rt of mel is 13.83 min
Rt of benzothiazine is 4.96 min
Rt of 5-methyl-2-aminothiazole is 3.86 min.

The composition of the mobile phase was adjusted after varying the methanol to the buffer ratio. Further increase in the buffer prolong the retention time and increased tailing. Acetate buffer pH 4.3 was found to ensure complete resolution of the three peaks. Variable columns were tried such as hypersil ODS 5 μm (250 × 4.6 mm), micro bondapack 5 μm (300 × 4.6 mm), spherisorb ODS (250 × 4.6 mm) 5 μm, all showed good resolution and elution time within 20 min. Spherisorb was used for method validation as it showed minimum elution time.

By applying the proposed method a linear correlation was obtained between area under the peak and the concentration in the range 100–500 μg ml^{-1} from which the linear regression equation was calculated.

$$\text{Area} = 33.2 \times \text{concentration} - 29.33 \quad r = 0.9990$$

The %RSD of the slope and the intercept for the linearity study was 0.11 and 0.09, respectively.

The proposed method is valid for the determination of mel in the presence of 10–90% of the degradation products with mean recovery 99.09 ± 0.38.

All solutions of mel and it's degradation products are freshly prepared to ensure stability of analyte in solution.

3.2. Methylene blue complexation

Mel reacts with basic dyes like MB in the presence of phosphate buffer, forming a highly coloured neutral complex which is extractable into chloroform (Fig. 2). At the specified pH value neither the drug nor the dye was extracted into chloroform.

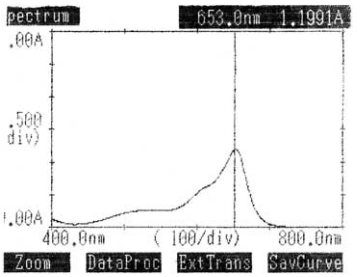

Fig. 2. Spectrophotometric spectrum of the formed complex with MB.

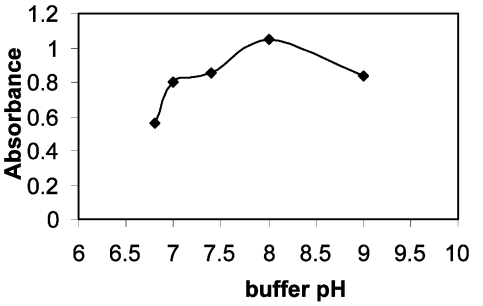

Fig. 3. Effect of pH on the formed mel–MB complex.

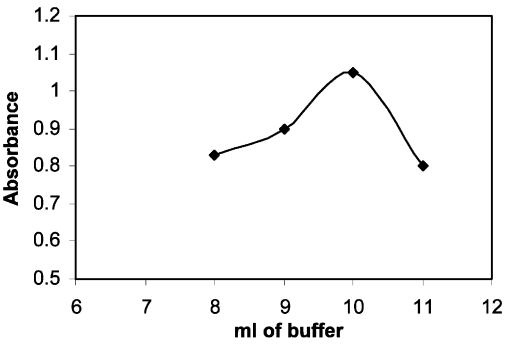

Fig. 4. Effect of ml of buffer pH 8 on the absorbance of the formed mel–MB complex.

3.2.1. Optimisation of variables

Investigation was carried out to establish the most favourable conditions for the reaction.

3.2.1.1. pH of the buffer. Phosphate buffers of different pH values were tested. Maximum absor-

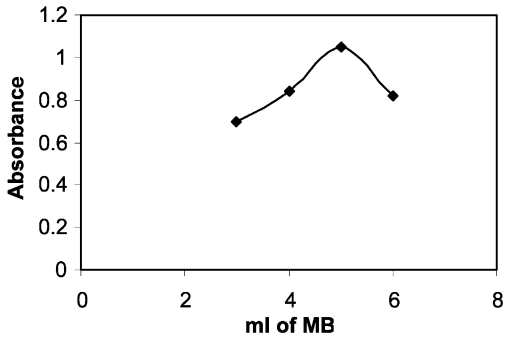

Fig. 5. Effect of ml of MB on the absorbance of mel–MA complex.

bance was obtained using phosphate buffer pH 8 (Fig. 3).

3.2.1.2. Volume of buffer pH 8. Ten milliliters phosphate buffer showed highest absorbance (Fig. 4) as it achieves complete ionisation of the drug and MB, a further increase in the buffer volume showed a decrease in the absorbance which may be attributed to shielding effect to the formed ions.

3.2.1.3. Volume of 0.1% w/v MB. Five milliliters of 0.1% w/v MB showed maximum absorbance (Fig. 5).

3.2.1.4. Extracting solvent. Several solvents such as chloroform, toluene, hexane were tested. The formed complex is extracted only in chloroform.

3.2.1.5. Shaking time for extraction. One minute was sufficient for complete extraction, a further increase in the extraction time did not affect the absorbance.

3.2.1.6. Phase volume ratio. Varying the ratio of organic to aqueous phase in the main extraction showed maximum absorbance when the ratio of organic to aqueous was 25:15.

3.2.1.7. Stability of the formed complex. The complex remained stable for 30 min.

3.2.1.8. The molar ratio of drug to reagent in the complex. It was determined by Job's method of

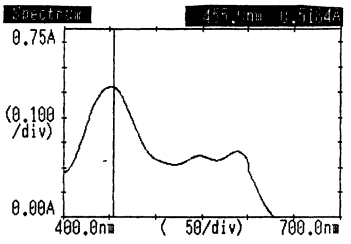

Fig. 6. Spectophotometric spectrum of the formed complex with DDQ.

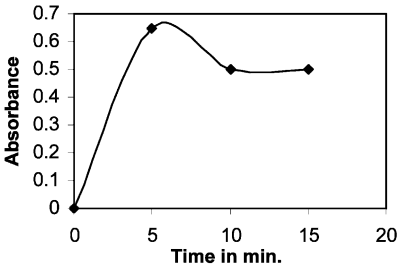

Fig. 7. Absorbance intensity of the formed DDQ anion as a function of time (in a boiling water bath).

continuous variation and was found to be 1:1 drug to reagent.

The linear regression equation for the MB complexation method is

$$\text{Absorbance} = 0.22 \times \text{concentration} - 0.043$$

$$r = 0.9980$$

The linearity range is over 1–5 μg ml^{-1}.

The %RSD of the slope and the intercept for the linearity study was 1.16 and 3.66, respectively.

3.3. DDQ method

π-Acceptors such as DDQ are known to react with a variety of electron donors forming a charge transfer complex which dissociate forming an intense orange red coloured ion referred to DDQ anion [15] (Fig. 6).

3.3.1. The reaction conditions were optimised as follows

3.3.1.1. The effect of heating time in a boiling water bath. Heating in a boiling water bath for 5 min showed highest absorbance (Fig. 7).

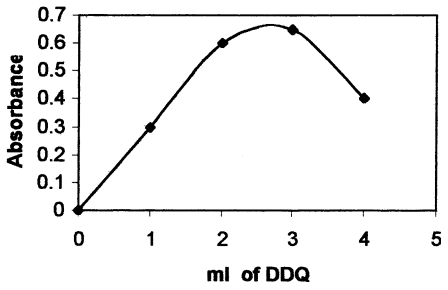

Fig. 8. Effect of the volume of DDQ on the absorbance.

3.3.1.2. Amount of the reagent. Three milliliters of DDQ 0.1% w/v in acetonitrile showed highest absorbance (Fig. 8).

3.3.1.3. Stability of the formed colour. The colour was stable for 1 h.

3.3.1.4. The molar ratio of drug to the reagent in the formed complex. It was determined by Job's method of continuous variation and was found to be 1:2 drug to reagent.

The linear regression equation of DDQ method is

$$\text{Absorbance} = 0.004100 \times \text{concentration} - 0.004433$$

$$r = 0.9970$$

The linearity study is over concentration range 40–160 μg ml^{-1}.

The %RSD of the slope and the intercept for the linearity study is 1.24 and 2.44, respectively.

3.4. Validation of the proposed methods

3.4.1. Precision and accuracy

The accuracy and reproducibility of the results in terms of percentage recovery of pure samples of intact drug analysed by the proposed methods are shown (Table 1). Statistical comparison between the results of the proposed and official methods showed no significant difference (Table 2). Intra-day precision and accuracy of the proposed methods were evaluated by assaying freshly prepared solutions in triplicates at three different concentrations for the three methods. Inter-day

Table 1
The result of the proposed and official methods in determination of mel in pure drug

Experiment number	HPLC method[a] (found %)	MB method[a] (found %)	DDQ method[a] (found %)	Official method[a] (found %)
1	100.67	101.00	101.8	98.96
2	100.36	99.45	101.4	98.32
3	100.21	98.70	100.33	99.65
4	99.25	98.30	99.60	99.93
5	100.18	98.14	99.50	100.12
Mean	100.13	99.12	100.53	99.40
%RSD	0.53	1.18	1.03	0.75

[a] Each result is the average of three experiments.

Table 2
Comparison between the results of the official and proposed methods in determination of pure samples

	HPLC method	MB method	DDQ method	Official method
Range of concentration	100–500 μg ml^{-1}	1–5 μg ml^{-1}	40–160 μg ml^{-1}	50–300 mg
Correlation coefficient	0.999	0.998	0.997	–
N	5	5	5	5
F (6.39)[a]	2	2.45	1.93	–
t (2.31)[a]	1.78	0.45	1.97	–

[a] Figures in parentheses represent corresponding tabulated values for F and t at $P = 0.05$.

Table 3
Intra- and inter-day assay variations of mel by the proposed methods

Proposed method	Intra-day assay variations			Inter-day assay variations		
[a]HPLC method	99.24	202.10	297.35	99.15	200.42	299.13
	98.75	200.52	301.42	99.07	200.86	301.45
	99.46	198.63	298.61	98.11	198.92	300.66
Mean	99.15	200.42	299.13	98.78	200.07	300.41
S.D.	0.36	1.74	2.08	0.58	1.02	1.18
%RSD	0.36	0.87	0.70	0.59	0.51	0.39
[a]MB method	0.9962	3.020	3.980	0.9938	2.990	3.990
	0.9830	2.970	4.010	0.9921	2.920	3.810
	1.0021	2.990	4.020	0.9853	3.000	3.880
Mean	0.9938	2.990	0.390	0.9904	2.970	3.890
S.D.	0.0098	0.025	0.021	0.0045	0.044	0.091
%RSD	0.99	0.84	0.54	0.45	1.48	2.34
[a]DDQ method	48.91	81.01	119.15	49.43	80.71	119.39
	49.53	80.42	119.01	48.91	79.50	120.11
	49.84	80.71	120.02	49.70	79.83	120.42
Mean	49.43	80.71	119.39	49.35	80.01	119.97
S.D.	0.47	0.30	0.55	0.40	0.63	0.53
%RSD	0.95	0.37	0.46	0.81	0.79	0.44

[a] Each concentration is in μg ml^{-1}.

Table 4
Comparison between the official method and proposed HPLC method in determination of mel in the presence of its degradation products

Sample number	% degradation	HPLC method (% recovery)	Official method (% recovery)
1	10	98.93	108
2	30	99.35	119
3	50	99.62	125
4	70	98.85	150
5	90	98.72	206
Mean	–	99.09	–
%RSD	–	0.38	–

precision and accuracy were evaluated by assaying freshly prepared solutions in triplicates for three different days (Table 3).

3.4.2. *Specificity*

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences (degradation products, related substances, excepients). Specificity of the HPLC method was checked by adding all known degradation products to pure mel samples and the response of the analyte was measured giving accurate and precise results. Applying the official method for the determination of mel in the presence of its degradation products showed unacceptable results (Table 4). Application of all the proposed methods to mel pharmaceutical formulations showed no interference from excepients

Table 5
Application of the proposed methods in the determination of pharmaceutical preparations

Pharmaceutical formulations	HPLC method		MB method		DDQ method	
	% Recovery of the test ± %RSD	Standard addition ± %RSD	% Recovery of test ± %RSD	Standard addition ± %RSD	% Recovery of test ± %RSD	Standard addition ± %RSD
Mobitil 15 mg/tablet	99.83 ± 0.48	99.35 ± 0.51	98.38 ± 0.69	98.93 ± 0.82	97.95 ± 0.70	99.18 ± 0.51
Mobitil 7.5 mg/tablet	98.05 ± 0.20	99.73 ± 0.40	97.14 ± 0.47	99.16 ± 0.62	98.91 ± 0.47	99.08 ± 0.62
Mobic 15 mg/tablet	99.45 ± 0.68	99.24 ± 0.59	98.02 ± 0.42	98.68 ± 0.51	96.57 ± 0.50	99.02 ± 0.51
Mobic 7.5 mg/tablet (15 mg/supp)	98.88 ± 0.43	99.73 ± 0.62	99.54 ± 0.72	99.11 ± 0.30	98.78 ± 0.30	99.73 ± 0.41
Mobitil suppository (15 mg/supp)	98.85 ± 0.56	99.30 ± 0.39	98.34 ± 0.49	99.24 ± 0.50	98.67 ± 0.65	99.11 ± 0.62
Mobic suppository (15 mg/supp)	98.94 ± 0.69	99.80 ± 0.46	98.24 ± 0.63	97.78 ± 0.31	98.03 ± 0.86	99.34 ± 0.68
Mexicam suppository	99.29 ± 0.91	99.66 ± 0.41	98.61 ± 0.81	100.11 ± 0.66	98.91 ± 0.64	99.90 ± 0.50

and good results were obtained. Standard addition technique was applied to ensure the accuracy of the proposed methods (Table 5).

3.4.3. Sensitivity

The proposed methods were capable to determine mel at low concentration up to 1 μg ml^{-1} for the MB method, 40 μg ml^{-1}, for the DDQ method and 100 μg ml^{-1} for the HPLC method.

3.4.4. Stability

Analysing commercial formulations by the proposed HPLC method kept at room temperature on the laboratory bench or in the refrigerator showed no degradation products only samples under stress conditions showed degradation.

3.4.5. Limit of detection and limit of quantitation

Limit of detection (LOD) represents the concentration of analyte that would yield a signal-to-noise ratio of three [16]. LOD is 3.65 μg ml^{-1}, 2.7 ng ml^{-1}, 3.38 μg ml^{-1} for the HPLC, MB and DDQ methods, respectively. The limit of quantitation (LOQ) represents the concentration of analyte that would yield a signal-to-noise ratio of ten [16]. LOQ 12.16 μg ml^{-1}, 9.09 ng ml^{-1} and 11.279 μg ml^{-1}, respectively.

4. Conclusion

Three methods for quantitative determination of mel in both pure form and pharmaceutical formulation were presented. The first method is HPLC it is stability indicating method and capable of differentiating between mel and its two degradation products, also it is accurate with less intra- and inter-day variations than the published method [8] and better accuracy than the reported method [12]. The other two methods are spectrophotometric, they have the advantages of simplicity, low cost, availability of equipment and sensitivity. They show better percentage recovery than the reported spectrophotometric method [4]. All the proposed methods are more sensitive than the official method [3]. The results obtained on using the three methods are compared statistically with those obtained on using the official method.

No statistical difference in determination of intact pure drug between them. However unacceptable results were obtained on applying the official method in the presence of degradation products. Validity was confirmed by applying standard addition technique where the percentage recovery of added standard was about the same as the pure substance.

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