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QUANTIFICATION ASSAY OF METHOXSALEN FROM BULK DOSAGE FORM AND IT'S ALKALINE STRESS CONDITION BY UV-SPECTROMETRY METHOD

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ABSTRACT

Rapid, specific and economic UV spectrophotometric method has been developed to determine the methoxsalen content in bulk and pharmaceutical dosage formulations. At a pre-determined λ max of 247nm, it was proved linear in the range of 1.0–12.0 mg/mL and exhibited good correlation coefficient (R^2=0.996). This method was successfully applied to the determination of methoxsalen content in marketed brand(Melanocyl R^2-0.982, Meladerm R^2-0.971) and the results were in good agreement with the label claims. Methoxsalen is a photosensitizing agent which has a chemical structure susceptible to degradation. The obtained results proved that the method can be employed for the routine analysis of methoxsalen in bulks as well as in the commercial formulations.

KEYWORDS: Methoxsalen, UV-Spectrscopy and Marketed preparation, Alkaline stress condition.

INTRODUCTION

Methoxsalen is chemically designated as 9-methoxy-7H-furo-(3, 2-g)-1-benzopyran (7) one. It is a photosensitizer that greatly increases the skin reactivity to long wavelength ultraviolet radiations (200 to 400 nm).

It is indicated for the treatment of psoriasis, eczema and to repigment the vitiliginous areas of the skin in conjunction with controlled doses of ultraviolet A (200-400 nm) or sunlight.[1]

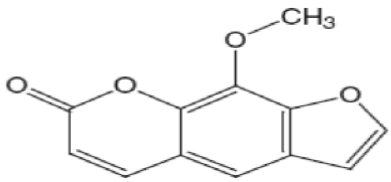


Fig No. 1 Structure of Methoxsalen.

Several HPLC assay methods have been reported for the determination of methoxsalen.[2–6] Various analytical procedures for methoxsalen have been reported either alone or in combination with other drugs. Methoxsalen has been determined by LC-MS.[7-8] Literature survey revealed that various analytical methods such as high performance thin layer chromatography (HPTLC)[9] and conductometry[10] have been reported for the estimation of methoxsalen.[11] The developed method was as per the guidelines of International Conference on Harmonization (ICH)[12] and demonstrated excellent specificity, linearity, precision and accuracy for methoxsalen.

MATERIALS AND METHODS

Apparatus

A ShimadzuUV–visiblespectrophotometer (UVmini-1600, Shimadzu Corporation, Kyoto, Japan) was used for all absorbance measurements with matched quartz cells.

Materials

All chemicals and reagents were of analytical or HPLC grade. Methoxsalen powder was provided by Inga Pharmaceuticals Ltd, Mumbai. This was used as the reference standard.

Marketed Formulation

Melanocyl tab and meladerm tab was purchased from an open market for this study, which contains methoxsalen10mg.

Preparation of Standard Stock Solutions

10 mg of methoxsalen working standard was weighed accurately and transferred to a 10 ml Volumetric flask. Solution was sonicated and diluted up to the mark with ethanol.

Preparation of Working Standard Solutions

The prepared stock solution was further diluted with distilled water to get working Standard solutions of 10 ppm of the drug. To construct Beer's law plot for pure drug, different Aliquots of the drug were taken and diluted to 10 ml with distilled water.

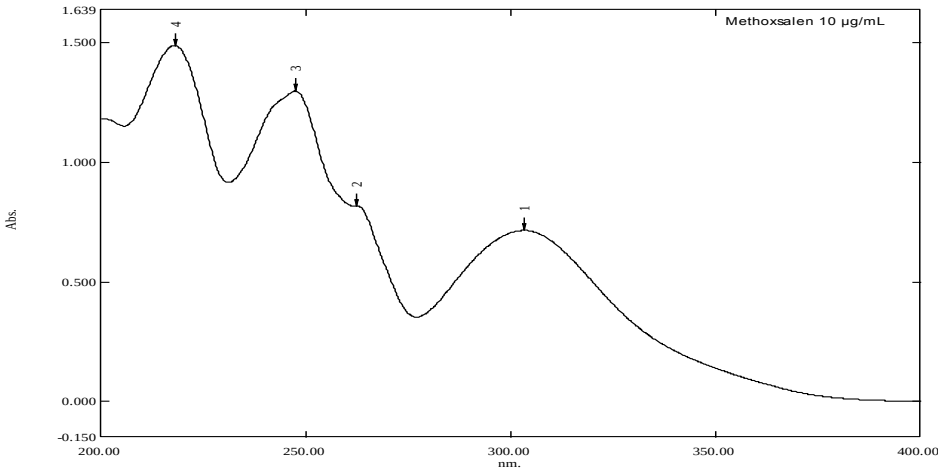
For Formulation

The average weight of the tablets were determined by weighing 20 tablets and powdered. Tablet powder equivalent to 10 mg of methoxsalen was weighed and transferred to a 100 ml volumetric flask. About 60 ml of ethanol was added and sonicated for 15 minutes complete dissolution of drugs, made up to the volume with ethanol and filtered through filter paper. Dilutions were made with ethanol to attain a concentration of 10 µg/ml and spectra was recorded. The average weight of the tablet (Meladerm) was found to be 102mg and for (Melanocyl) 202 mg. The procedure was kept same for both brands.

Stress Condition

Alkali degradation: - For study in alkaline condition, aliquot quantity of drug was weighed and transfer in 100 ml volumetric flask. To this 2ml of 0.1M, 1 M, 5M NaOH, was added and diluted to 50 ml with water. This solution was refluxed at 80c for 4hr. cool to room temperature make valume upto 100 ml with water solution was further diluted to give 10ug/ml and then scan over a range of 400-200 nm keeping solvent as blank analyzed by U-V_Spectrophotometer 1600.

Scanning spectra for methoxsalen in water over range of 400 to 200 nm against water as blank



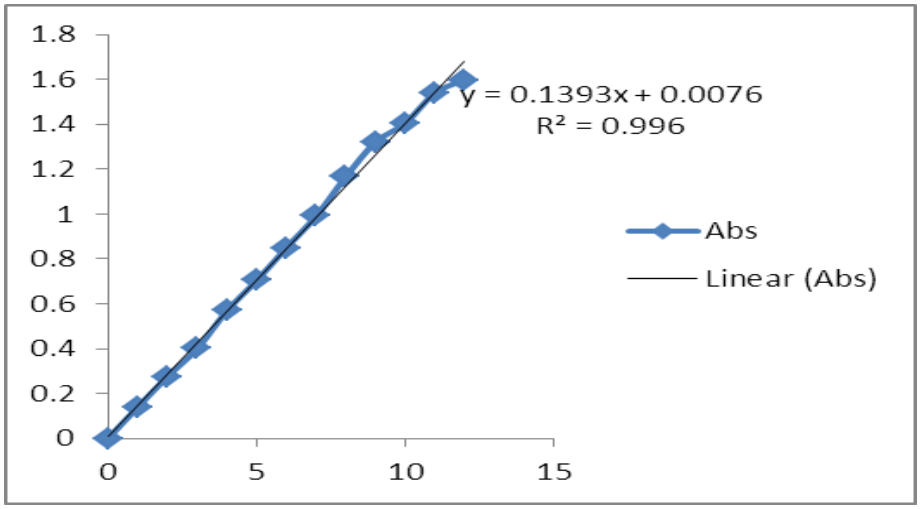
RESULT AND DISCUSSION

Method development and optimization

Methoxsalen is almost insoluble in aqueous medium and freely soluble in organic solvents like ethanol, chloroform and acetonitrile. During the development phase, the use of a few milliliters of chloroform and ethanol with water as the diluents resulted in preferable outcome in UV analysis. The pre-determined wavelength of maximum absorption (λ max) was 247nm.

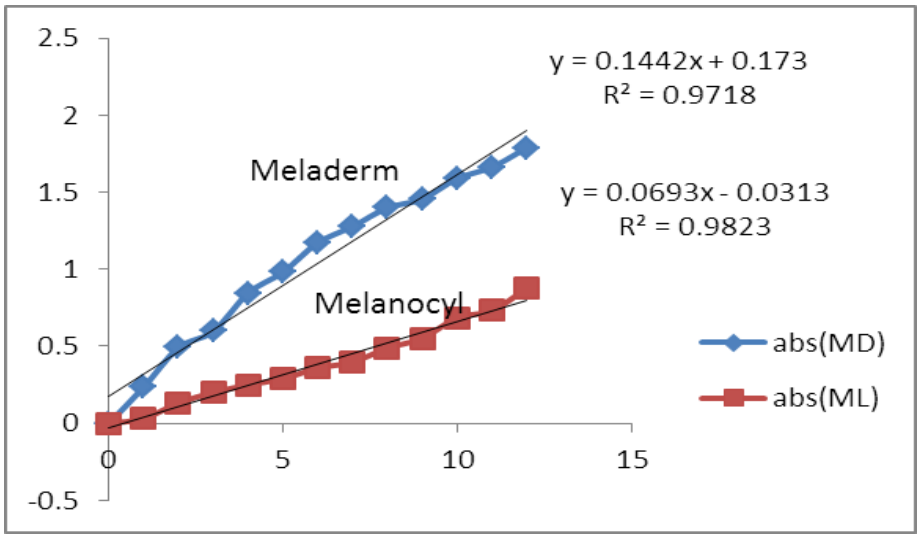
Calibration curve for Methoxsalen

Sr. no	Concentration (µg/ml)	Absorbance at 247nm
1	1	0.140
2	2	0.276
3	3	0.401
4	4	0.569
5	5	0.704
6	6	0.850
7	7	0.991
8	8	1.166
9	9	1.321
10	10	1.405
11	11	1.540
12	12	1.598

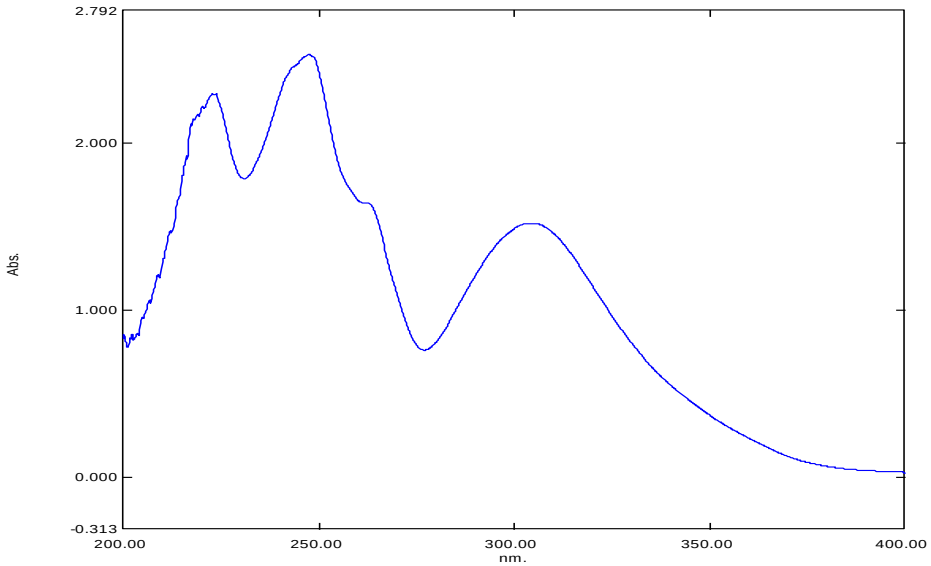


Calibration curve for marketed formulation

Concentration (µg/ml)	Absorbance at 247nm(MD)	Absorbance at 247nm(ML)
1	0.235	0.032
2	0.491	0.132
3	0.598	0.201
4	0.845	0.25
5	0.982	0.290
6	1.172	0.359
7	1.278	0.398
8	1.399	0.489
9	1.456	0.552
10	1.589	0.685
11	1.663	0.732
12	1.789	0.876

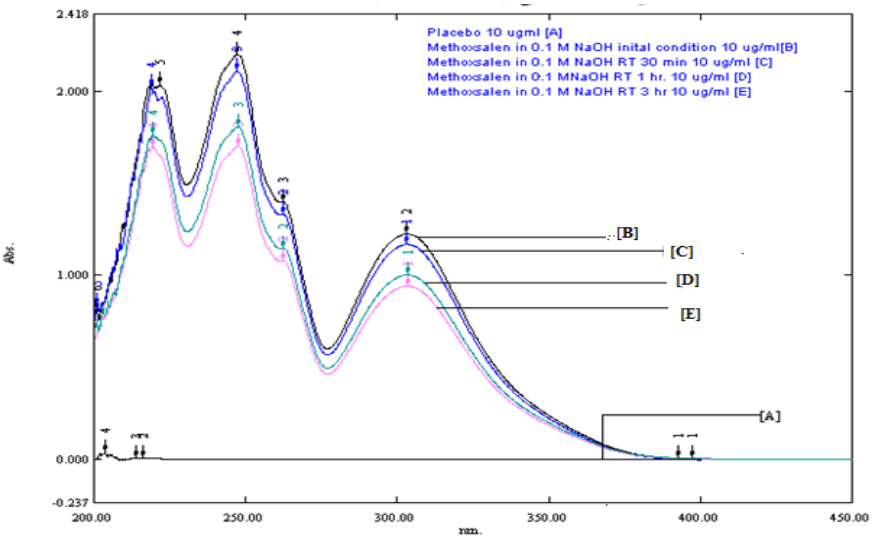


Scanning spectra for Marketed preparation containing methoxsalen in water over range of 400 to 200 nm against water as blank (10ug/ml)



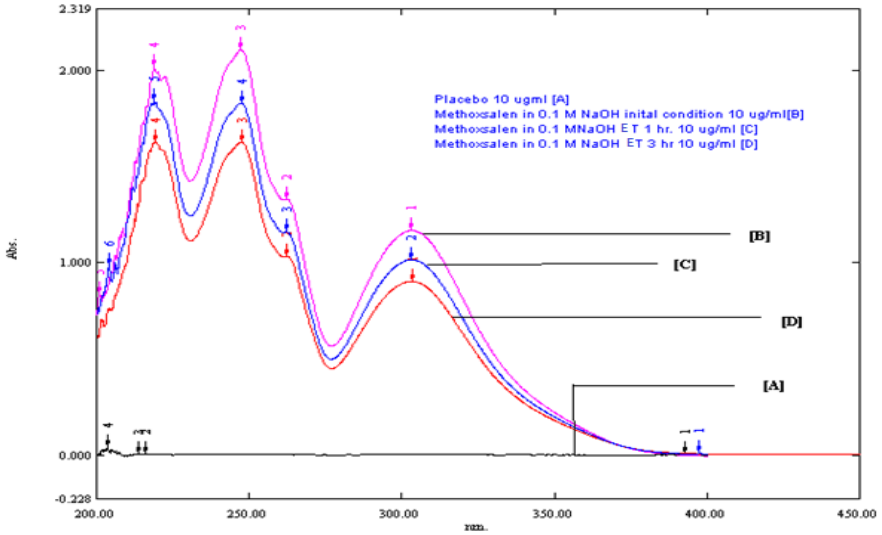
Methoxsalen in Alkaline medium (0.1M NaOH) @Room Temperature

Sr. n0	Wavelength	Absorbance @ RT			
		0 hr	30 min	1 hr	3 hr
1)	397			0.007	NA
2)	303.20	1.166	0.940	1.223	1.001
3)	262.40	1.330	1.075	1.397	1.145
4)	247.00	2.107	1.700	1.675	1.535
5)	218.80	2.019	1.697	2.032	1.764
6)	201.00	0.832	NA	0.808	NA
7)	@ 247 nm		19 %	21%	27%



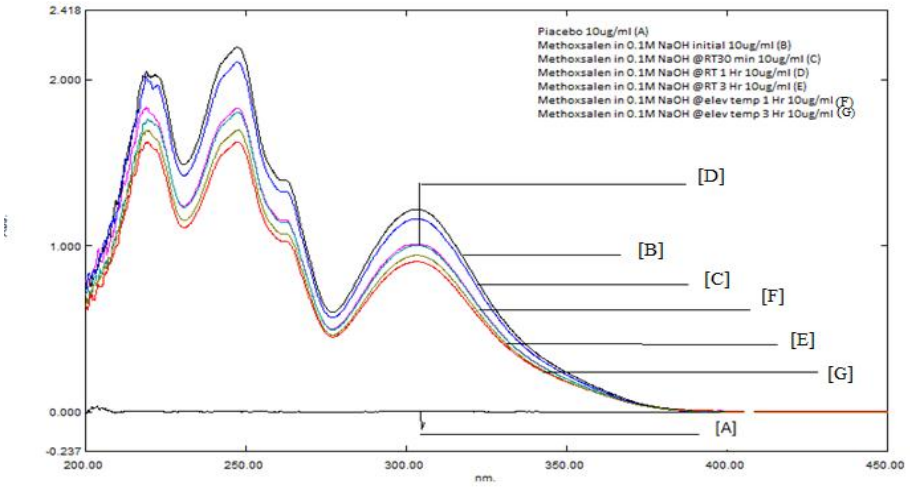
Methoxsalen in Alkaline medium (0.1M NaOH) @Elevated Temperature

Sr no	Wavelength	Absorbance @ Elevated Temperature			
		0 hr	30 min	1 hr	3 hr
1)	397			0.009	
2)	303.20	1.166	0.940	1.014	0.902
3)	262.40	1.330	1.075	1.159	1.032
4)	247.00	2.107	1.669	1.590	1.492
5)	218.80	2.019	1.697	1.837	1.626
6)	201.00	0.832	NA	0.982	1.625
7)	@ 247 nm		21 %	25%	30%



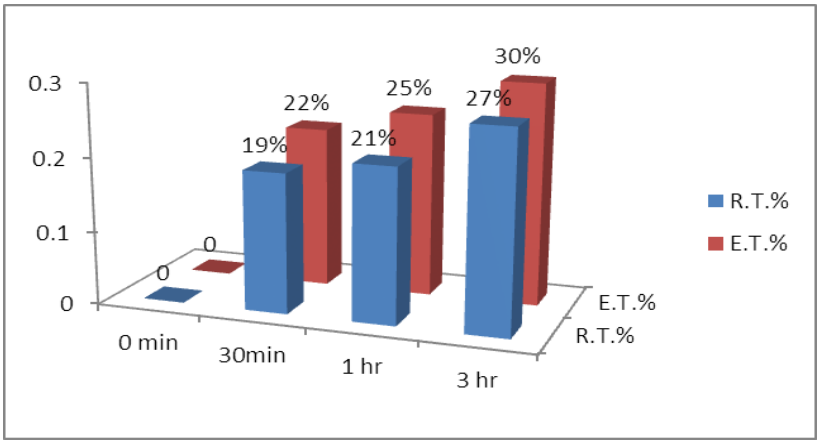
Methoxsalen in Alkaline medium (0.1M NaOH) @ Room Temp &Elevated Temperature

Sr no	Wavelength	Absorbance @ RT				Absorbance @ elevated temperature			
		0 hr	30 min	1 hr	3 hr	0 hr	30 min	1 hr	3 hr
1)	397	NA	NA	0.007	NA	NA	NA	0.009	NA
2)	303.20	1.166	0.947	1.223	1.001	1.166	0.914	1.014	0.902
3)	262.40	1.330	1.079	1.239	1.145	1.330	1.042	1.159	1.032
4)	247.00	2.107	1.700	1.675	1.535	2.107	1.699	1.590	1.492
5)	218.80	2.019	1.685	2.032	1.764	2.019	1.661	1.837	1.626
6)	201.00	0.832	NA	0.808	NA	0.832		0.982	1.625
7)	@ 247 nm		19 %	21%	27%		22 %	25%	30%



Graphical presentation of alkaline degradation studty for room temp & elevated temp

Stress Condition	Time (Hours)	% Degraded product @ Room Temp	% Degraded product @ Elevated Temp
Alkaline Stress Condition	0 hr condition	0 %	0%
Alkaline Stress Condition	Half hr condition	19 %	22%
Alkaline Stress Condition	One hr condition	21%	25%
Alkaline Stress Condition	Three hr condition	27%	30%



CONCLUSION

The results and the statistical parameters demonstrate that the proposed UV spectrophotometric method is simple, accurate and precise. Therefore, this method can be used for the determination of methoxsalen either in bulk or in the dosage formulations without interference with commonly used excipients and related substances. pharmaceutical samples reported in this work is simple, fast, inexpensive, and thus appropriate for routine quality control analysis of the active drug in the laboratories of hospitals, pharmaceutical industries and research institutions. It should also be suitable for developing countries. This demonstrates that the developed method was specific and stability-indicating**.** The ICH not provided any formal guidance. As a success to degradation study absolutely relies on skillfulness on researcher, it is indispensable to understand the precise.

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