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Original Article

APPLICATION OF HPTLC FOR THE QUALITATIVE AND QUANTITATIVE ANALYSIS OF AVOBENZONE, OXYBENZONE, OCTINOXATE IN SUNSCREEN CREAM

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ABSTRACT

Objective: This paper presents the development of an improved method for the Qualitative and Quantitative analysis of Avobenzone(AVO), Oxybenzone(OXY) and Octinoxate(OCTI) using high-performance thin-layer chromatography (HPTLC) with densitometric detection.

Methods: Separation was performed on silica gel 60F254plates and RP 60F254plates. The mobile phase is comprised of Actonitrile:Water(18:2) for RP 60F254 plate and Cyclohexane:n-Hexane:Acetone:Diethyl Ether(14:1:2:2) then both plate were scanned at 254nm and 366nm.

Results: The Rf values were 0.55,0.62 for AVO,OCTI respectively separated on silica gel 60F254plates and 0.63 for OXY separated on RP 60F254plate. By using this method recovery of all three sunscreen agents were 92.7%,99.47%,102.4% of OXY,AVO and OCTI respectively.

Conclusion: The developed HPTLC method combined with densitometry was found suitable for Qualitative and Quantitative determination OXY,AVO and OCTI from sunscreen cream without any interference of excipients.

Keywords: Densitometry, HPTLC, Sunscreen, Qualitative and Quantitative analysis.

INTRODUCTION

A sunscreen cosmetic could be defined as "any cosmetic product containing UV filters in its formulation in order to protect the skin from t the solar deleterious UVlight, avoiding or minimizing the damage that this radiation might cause on human health" [1]. Extraterrestrial sunlight includes X-ray, ionizing, ultraviolet (UV), visible and infrared radiation, and radio waves. Oxybenzone, Octinoxate and Avobenzone are also called as **UV filters** as they prevent damage to the skin from UV radiation. UV spectrum is divided into three bands. UVA (320-400nm), UVB (290-320nm) and UVC (200-290nm).

UVA radiation is more harmful then other two. UVB radiation is fully absorbed by the stratum corneum and the top layers of the epidermis, whereas up to 50% of incident UVA radiation penetrates Caucasian skin deep into the dermis. [2]

Oxybenzone is chemically (2-hydroxy-4-methoxyphenyl)-phenyl - methanone with a molecular weight228.24g/mol. It is an important compound in organic photochemistry and organic synthesis. It is a white cryslline powder which is insoluble in water and having a melting point 49^0 C. It is a derivative of benzophenone. Benzophenones absorb UVB and some UVA (to approximately 360 nm, with a peak at 290 nm). The most popular benzophenone and one of the most common sunscreen ingredients is benzophenone-3 or oxybenzophenone. It has been isolated in the blood and urine ofhumans [3-5] after topical application. Compared with other UV filters, benzophenone-3 is the most bioavailable following topical application; however, this bioavailability is not of toxicologic concern. [3, 6] Moreover, it has the highest reported incidence of photodermatitis. The photomutagenic properties of these compounds might be a contributing factor to the increased melanoma incidence that has been found in sunscreen users. Other possibilities include consequent overexposure to sun without UVA protection and vitamin D deficiency from overuse of sunscreen.

Octinoxate is chemically ethyl hexyl methoxy cinnamate. It is frequently used in combination with other UVB absorbers to achieve high SPF values in the final product. Topical application of OMC is tolerated well: skin irritation is almost negligible, and photocontact dermatitis is rare. Upon exposure to sunlight, octinoxate degrades into a photoproduct with less UV-absorbing ability. Several studies suggest ways to improve the photostability of cinnamate Encapsulation of ethylhexyl*p*methoxycinnamate into nanoparticles results in a reduction of the photodegradation [7]. Animal studies indicate that octyl methoxycinnamate may produce hormonal (estrogen-like) and possibly other adverse effects. Whether typical human use of octyl methoxycinnamate may cause such effects is unclear.

Avobenzone is chemically 4-tertbutyl- 4'-methoxy-dibenzoyl methane with a molecular weight 310.39. It is insoluble in water but freely soluble in organic solvent like methanol. Avobenzone having a phenyl ketone group and sterically hindered group in a molecule. It absorbs wider range of UV wavelength. It absorbs UVA (320-400nm, and it is associated with long term skin damage) and UVB (290-320nm, that causes sunburn rays).It is one of most effective sun-screen ingredient. The analytical control of the sunscreen cosmetics is necessary since the content of UV filters in the final product is related to its sun protection efficacy that is usually claimed by the labeled sun protection factor (SPF).

The **sun protection factor** is a measure of the ability of a sunscreen to protect against erythema[8] which is thus primarily a measure of UVB protection. The SPF is a ratio of the dose of UV radiation required to produce a minimal erythema 24 hours after exposure in sunscreen-protected skin to the dose required to produce the same degree of erythema in unprotected skin. In other words: the SPF number defines how long you can stay in the sun before getting burnt. If it normally takes you 20 minutes in the sun before you get burned, an SPF 15 product will let you stay 15 times longer in the sun: 20 min x 15 (SPF) = 300 min (5 hours).

Several methods have been reported for analysis of sun-screeningredients by second order spectroscopy, flow injection isodifferential derivative spectroscopy, UV spectroscopy and by UPLC. Several methods also been reported for analysis of other sun-screen ingredient like Homosalate, Octyl Dimethyl PABA, Octyl Salicylate, Camphor, Phenyl benzimidazole sulphonic acid along with Oxybenzone and Octinoxate by RPHPLC and it will take about 100min runtime. But no method has been reported for analysis and separation of Oxybenzone, Octinoxate and Avobenzone in sun-screencream by HPTLC method.[9]

MATERIALS AND METHODS

Chemicals

All the Chemical and Reagent used were of analytical grade and were provided by Anchrome Testlab Pvt. Ltd. Mulund(E), Mumbai, India. Chemical Sunscreen Agents i.e Avobenzone, Oxybenzone, Octinoxate were obtained from Ajanta pharma.Pvt.Ltd.Kandivali, Mumbai.

Instrumentation

The instrument used in the present study was CAMAG HPTLC system comprising CAMAG Linomat IV automatic sample applicator. The HPTLC system consisted of Linomat IV auto sprayer connected to a nitrogen cylinder a twin through glass chamber Silic Gel $60F_{254}$ TLC plates (20 X 10 cm). RP-18 F_{254s} TLC Plate (20X 10 cm) were used as stationary phase. Using Deutorium D_2 lamp, TLC plate is scan using CAMAG TLC scanner III in Remission-Fluroscence mode at 366 nm using Mercury Hg lamp.

Chromatographic conditions

First 9 spots applied are of standard solution of Avobenzone, Oxybenzone,Octinoxate then next 5 spots applied are of cream sample. The space between two bands was 12.3 mm. The slit dimension was kept at 6.00 x 0.45 mm, Micro and 20mm/s scanning speed was employed.

The mobile phase consisted of Acetonitrile: Water (18:2)for RP plate and Cyclohexane: Diethyl ether: n-Hexane: Acetone(14:2:1:2) for Normal plate and 20 ml of mobile phase was used per chromatography. Linear ascending development was carried out in 20x10 cm twin trough glass chamber saturated with mobile phase.

Standard solution preparation

Pure standards of Oxybenzone, Octinoxate & Avobenzone were prepared and analyzed under the optimized chromatographic conditions. Accurately weigh 10mg of

Oxybenzone, 20mg of Octinoxate and 10mg of Avobenzone and transfer into 10ml volumetric flask and volume was make up to mark with methanol.(Stock Solution) Sonicate it for 15-30 min.Take

1ml from it and diluted up to 10ml with methanol to obtain final concentration 100µgm/ml Oxybenzone, 200µgm/ml Octinoxate and 100µgm/ml Avobenzone. Filter it by using 0.2µ filter.

Sunscreen cream sample solution preparation

For preparing sample solution, 0.5gm of sample were weighted (each containing 2.3% of Oxybenzone, 3.8% of Octinoxate and 2.5% of Avobenzone) and transfer into 50 ml volumetric flask. They were dissolved in 50 ml of methanol to obtain final concentration 230µgm/ml Oxybenzone, 380µgm/ml Octinoxate and 250µgm/ml Avobenzone. Sonicate it for 30min. Filter it by using 0.2µ filter.

Standard and sample application On to TLC plate

Silic Gel $60F_{254}$ TLC plates (20X10cm), RP-18 F_{254s} TLC Plate (20 X 10 cm) TLC plates were used. This plate contain total 14 spots out of which 1 to 9 spots are standard solution (Avobenzone, Oxybenzone, Octinoxate) spots applied in bands containing volumes 1 µl,2 µl,3 µl,4 µl,5 µl,6 µl,7 µl,8 µl,9 µl respectively. And 10 to 14 spots are Sunscreen cream sample spots Applied in bands containing volume 3 µl.

RESULTS

For determination of Quantity of Oxyebnzone RP-18 F_{254s} TLC Plate (20 X 10 cm)(RP plate) is used and determination of Quantity of Avobenzone and Octinoxate Silic Gel $60F_{254}$(NP plate)TLC plates (20X10cm) is used. Mobile phase used in RP plate is Acetonotrile:water (18:2) and mobile phase used in NP plate is Cyclohexane: Diethyl ether: Acetone: n-Hexane (14:2:2:1). For determination of Oxybenzone RP plate is scanned at 254 nm(Figure1). For determination of Octinoxate NP plate is Scanned at 254 nm(Figure3). For determination of Avobenzone NP plate is scanned at 366nm(Figure2).

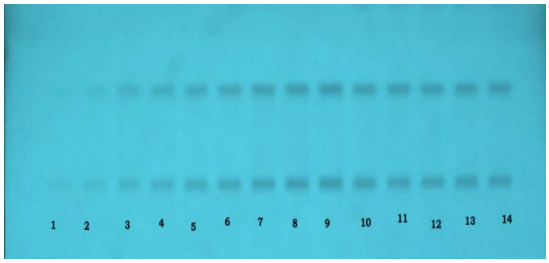

Fig.1: RP plate scan at 254 nm for determination of oxybenzone

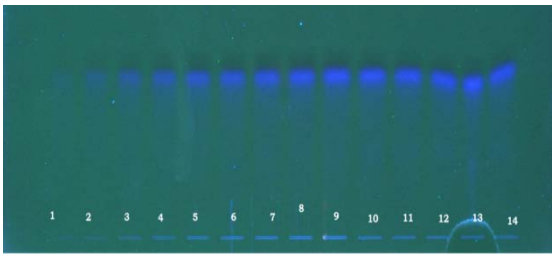

Fig. 2: NP plate scan at 366 nm for determination of Avobenzone

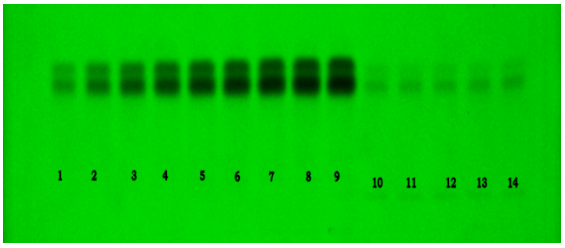

Fig. 3: NP plate scan at 254 nm for determination of Octinoxate

After scanning using densitometer Area of each band is plotted against respective concentration for each Sunscreen agent(Figure4,Figure5,Figure6). In the preparation of standard calibration cure concentration and its respective area for all 3 API and Area of Sample of sunscreen cream is given (Table1, Table2, Table 3) And then Area Obtained of the Sample of sunscreen is extrapolated onto this standard curve of Oxybenzone, Octinoxate and Avobenzone. Quantity obtained by single level calibration curve is mentioned in Table 4.

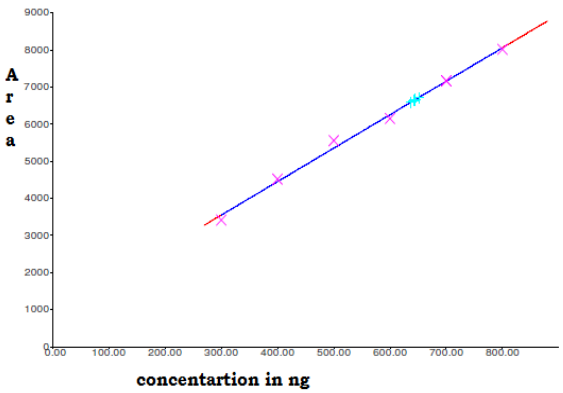

Fig. 4: Calibration curve of oxybenzone with extrapolated value of sample(in skyblue colour)

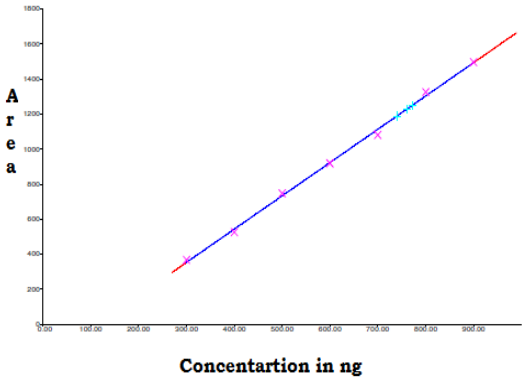

Fig. 5: Calibration curve of Avobenzone with extrapolated value of sample (in skyblue colour)

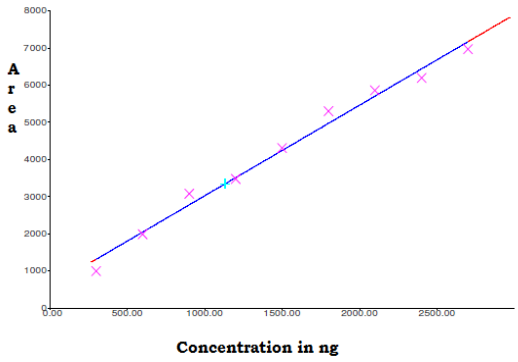

Fig. 6: Calibration curve of Octinoxate with extrapolated value of sample (in skyblue colour)

Table 1: Oxybenzone calibration curve data

S. No.	Concentration in ng	Rf value	Area
1	100	0.62	1125
2	200	0.62	2345
3	300	0.62	3385
4	400	0.64	4489
5	500	0.62	5524
6	600	0.62	6128
7	700	0.63	7139
8	800	0.63	7990
9	900	0.62	8785
10	Sample	0.62	6685
11	Sample	0.63	6675
12	Sample	0.62	6643
13	Sample	0.63	6698
14	Sample	0.62	6785

Table 2: Octinoxate calibration curve data

S. No.	Concentration in ng	Rf value	Area
1	300	0.62	970
2	600	0.62	1963
3	900	0.62	3058
4	1200	0.62	3457
5	1500	0.61	4285
6	1800	0.62	5281
7	2100	0.63	5837
8	2400	0.62	6174
9	2700	0.62	6951
10	Sample	0.62	3345
11	Sample	0.63	3326
12	Sample	0.62	3320
13	Sample	0.62	3321
14	Sample	0.61	3378

Table 3: Avobenzone calibration curve data

S. No.	Concentration in ng	Rf value	Area
1	100	0.55	184
2	200	0.56	258
3	300	0.55	366
4	400	0.55	524
5	500	0.56	746
6	600	0.56	917
7	700	0.55	1079
8	800	0.55	1324
9	900	0.55	1494
10	Sample	0.55	1254
11	Sample	0.55	1251
12	Sample	0.54	1187
13	Sample	0.56	1226
14	Sample	0.55	1198

Table 4: Result of Recovery study

S. No.	Name of Sunscreen Agent	Label claim of sunscreen scream	Ammount determine using HPTLC	Recovery (%)
1	Oxybenzone	2.3%	2.12%	92.17
2	Octinoxate	3.8%	3.78%	99.47
3	Avobenzone	2.5%	2.65	102.4

DISCUSSION

For the development of HPTLC method for the Quantitative analysis of the Avobenzone, Oxybenzone, Octinoxate in Sunscreen cream required two plates(RP plate and NP plate). RP plate is containing the C18 chain TLC plate. So that compound which more non polar (oxybenzone) get separated at higher Rf value 0.63. using RP plate avobenzone and Octinoxate were coming at same Rf value that is 0.34 due to the structural similarity between them. so to separate them NP plate is used. Using NP plate avobenzone and Octinoxate is get separated at different Rf value that is 0.56 for avobenzone and 0.62 for octinoxate.

But in NP plate Avobenzone and Oxybenzone were coming at same Rf value. But Avobenzone is showing fluroscence at 366nm and oxybenzone, Octinoxate are not showing fluroscence at 366nm. So that Quantitative analysis of Avobenzone is done on NP plate at 366nm.

HPLC methods would consume not less than 100 ml per runs of similar number of samples. If we consider the time from sample preparation to densitometric evolution for one plate, the new method takes an average of 1 h, whereas HPLC methods would generally take more than 2 h for the same number of samples. It is cheap and quick as compare to HPLC method.

CONCLUSION

The developed HPTLC method combined with densitometry was found suitable for determination of Avobenzone, Oxybenzone and Octinoxate as a bulk drug in topical formulation like sunscreen cream without any interference from excipients. Results of experiment shown that this method can be used for Quantitative and Qaualitative analysis of Avobenzone, Oxybenzone, Octinoxate. Its advantages are low cost of reagents, speed, and simplicity of sample treatment.

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGEMENT

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