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Combined RP-HPLC methodology for the determination of Dexpanthenol, its impurities and preservatives in topical formulations

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ABSTRACT: Background: Dexpanthenol is a stable alcoholic analog of pantothenic acid. It is used in topical applications. It has good skin penetration. This drug attains high local concentrations when administered in an adequate vehicle, such as water-in-oil emulsions. **Aim:** To develop a combined HPLC methodology for topical formulation of Dexpanthenol, its related impurities and preservatives in a single run. **Method:** The method was developed using a Thermo Scientific HPLC system (Ultimate 3000) with a BDS Hypersil C_{18} column (4.6 × 250 mm I.D., 5 µm) and gradient elution consisting of water and acetonitrile as the mobile phase. The detection wavelength was 210 nm for Dexpanthenol and 254 nm for methyl hydroxybenzoate and propyl hydroxybenzoate with an acquisition time of 40 min in which the related impurities along with Dexpanthenol and two preservatives were well separated. **Results:** The developed method was validated according to the ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the specified acceptance criteria. **Conclusion:** The proposed method was successfully applied to the topical dosage form for routine analysis

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INTRODUCTION:

Pantothenic acid, a member of the B complex vitamins (Vitamin B5), was discovered in 1931 by Roger J. Williams (1893 to 1988) during his studies on microbial growth factors [1,2]. The name pantothenic acid, should indicate its wide spread occurrence in nature [3,4]. In 1934, it was shown that pantothenic acid has a profound stimulating effect on cell proliferation in yeast which eventually led to the development of the first topical dexpanthenol preparation (Bepanthen^{TM}) [5,6].

Dexpanthenol (DPA) (CAS No. 81-13-0), (2R)-2,4-dihydroxy-N-(3-hydroxypropyl)-3,3-dimethyl butanemide is an alcoholic analogue of pantothenic acid, a member of the B complex vitamins (vitamin B5) [7]. Dexpanthenol is enzymatically oxidized to pantothenic acid, which is distributed into the tissues; mainly as coenzyme A [8]. Pantothenic acid is optically active, only the dextrorotatory isomer has biologic activity. Dexpanthenol is freely soluble in water and alcohol, practically insoluble in fats, and it is the most stable form of pantothenic acid in liquids. Dexpanthenol is used topically as an ointment, emulsion, or solution, at concentrations of 2 to 5 %, as an adjunct in the treatment of various skin and mucosal lesions [9-11]. It prevents skin irritation, stimulates skin regeneration and promotes wound healing. Two main directions in the use of topical dexpanthenol-containing formulations have therefore been pursued: as skin moisturizer/skin barrier restorer and as facilitator of wound healing [12,13]. Although discovered decades ago, the exact mechanisms of action of dexpanthenol have not been fully elucidated yet. With the adoption of new technologies, new light has been shed on dexpanthenol's mode of action at the molecular level [14-16]. It appears that dexpanthenol increases the mobility of stratum corneum molecular components which are important for barrier function and modulates the expression of genes important for wound healing [17-19].

For DPA, European Pharmacoepia (EP) and United States Pharmacopeia (USP) proposed a non-aqueous titration method due to the lack of chromophoric groups. Several methods have been reported on the analysis of panthenol using the pre-column derivatization technique using ninhydrin. Determination of panthenol in cosmetics and pharmaceutical formulations has been reported using the pre-column derivatization with fluorescamine by liquid chromatography technique and also using differential pulse voltammetry [20,21].

In the present work detailed as below, a liquid chromatographic method of analysis has been developed to simultaneously determine DPA, it impurities and preservatives which would be cost effective for routine testing purpose.

MATERIALS AND METHODS:

Chemicals and Reagents:

Dexpanthenol, Methyl Hydroxybenzoate and Propyl Hydroxybenzoate working standards were used available in Oman Pharmaceutical Products L.L.C. Topical formulation containing Dexpanthenol 5 % w/w was taken from the commercial batch manufactured at Oman Pharmaceutical Products L.L.C. HPLC grade Acetonitrile was procured from Merck Ltd. All other chemical reagents were of analytical grade.

Standard preparation:

About 80 mg of Methyl Hydroxybenzoate working standard, 8 mg of Propyl Hydroxybenzoate working standard and 100 mg of Dexpanthenol working standard were weighed and transferred in to a 100 ml volumetric flask, in to which 50 ml of methanol was added, sonicated to dissolve, diluted up to the mark with water and mixed well.

Further, 5 ml of the above stock solution was transferred into a 100 ml volumetric flask and diluted up to the mark with diluent. The obtained strength of Dexpanthenol, Methyl Hydroxybenzoate and Propyl Hydroxybenzoate were 50, 40 and 4 µg/ml.

Sample Preparation:

***Estimation of Preservative content*:**

Emptied the contents of an entire tube in a 20 ml syringe without a needle. The sample quantity equivalent to 50 mg of Dexpanthenol (Sample weight approximately 1 g) was weighed in to a 50 ml volumetric flask. To the flask, 25 ml of methanol was added. The contents of the flask were placed in a water bath maintained at 40 to 50 °C for 4 to 5 min to disperse the cream (swirled intermittently to get uniform dispersion). Then, it was sonicated for 10 min with intermittent swirling. The contents of the flask was cooled and diluted up to the mark with water and mixed well. The resultant solution was centrifuged at 5000 rpm for 10 min. Finally the solution was filtered layer through 0.45 µm Nylon membrane filter (SNY+GF Grade) (Cat. No: SNY045025H+GF). The prepared concentration of Methyl Hydroxybenzoate, Dexpanthenol and Propyl Hydroxybenzoate were 40, 1000 and 4 µg/ml respectively.

***Estimation of Dexpanthenol (Assay)*:**

The above 5 ml of filtered sample solution was transferred into a 100 ml volumetric flask, diluted to volume with diluent and mixed well. The solution was filtered through 0.45 µm Nylon membrane filter (SNY+GF Grade) (Cat.No:SNY045025H+GF). The concentration of Dexpanthenol solution was 50 µg/ml .

***Estimation of Dexpanthenol (Related substances)*:**

The above centrifuged sample solution was transferred into a 10 ml volumetric flask, diluted to volume with diluent and mixed well. The solution was filtered through 0.45 µm Nylon membrane filter (SNY+GF

Grade) (Cat.No:SNY045025H+GF). The concentration of Dexpanthenol solution was 500 µg/ml.

Dexpanthenol, its impurities and preservatives analysis:

The standard and test solutions were analyzed for content of Dexpanthenol, Methyl Hydroxybenzoate and Propyl Hydroxybenzoate by Chromatographic method as per the specification mentioned in Table 1. The mobile phase proportion is given in Table 2.

Table 1. Chromatographic conditions for analysis of Dexpanthenol.

Particulars	Specification
Instrument	HPLC
Column	250 × 4.6 mm, 5µm, BDS Hypersil C_{18} (Make : Thermo)
Mobile phase	Mobile phase-A: Water (100 %) Mobile phase-B: Acetonitrile (100 %)
Flow	1.0 ml/min
Detection	210 nm: Dexpanthenol 254 nm: Methyl Hydroxybenzoate and Propyl Hydroxybenzoate
Injection volume	20 µL
Sampler temperature	25 °C
Column oven temperature	25 °C
Run Time	40 min
Elution	Gradient
Diluent	Water : Methanol (1:1)

Table 2. The details of Mobile phase gradient programme.

Sl. No.	Time (min)	A %	B %
1	0	95	5
2	12	95	5
3	25	50	50
4	38	95	5
5	40	95	5

RESULTS AND DISCUSSION:

The developed method for determination of Dexpanthenol, its related impurities, methyl hydroxybenzoate and Propyl hydroxybenzoate was validated by using the following parameters.

System suitability (Assay, Preservative and RS):

For establishing the system suitability, the procedure described in the methodology was followed before starting the analysis. System suitability data has been presented in Table 3, 4, 4a and Fig 1 to 6.

Table 3. The system suitability data of Dexpanthenol.

Injection #	Area	Plate count	Tailing
1	63277	7215	0.92
2	63376	6807	0.93
3	63897	6954	0.94
4	63970	7083	0.93
5	64687	6723	0.91
6	64208	6194	0.89
Mean	63903	6829	0.92
SD	525.701	--	--
% RSD	0.8 %	--	--

SD – Standard deviation and RSD – Relative Standard deviation.

Table 4. The system suitability data of Methyl hydroxybenzoate (MHB).

Injection #	Area	Plate count	Tailing factor
1	734290	519688	1.09
2	734767	522139	1.05
3	742184	519971	1.05
4	740097	519688	1.11
5	750538	524322	1.06
6	747872	522139	1.05
Mean	741625	521325	1.07
SD	6662.55	--	--
% RSD	0.9 %	--	--

SD – Standard deviation and RSD – Relative Standard deviation.

Table 4a. The system suitability data of Propyl hydroxybenzoate (PHB).

Injection #	Area	Plate count	Tailing factor
1	64926	509508	1.07
2	64959	511282	1.08
3	65640	509508	1.06
4	65438	511282	1.04
5	66421	511282	1.06
6	66170	513065	1.07
Mean	65592	510988	1.06
SD	615.075	--	--
% RSD	0.9 %	--	--

SD – Standard deviation and RSD – Relative Standard deviation.

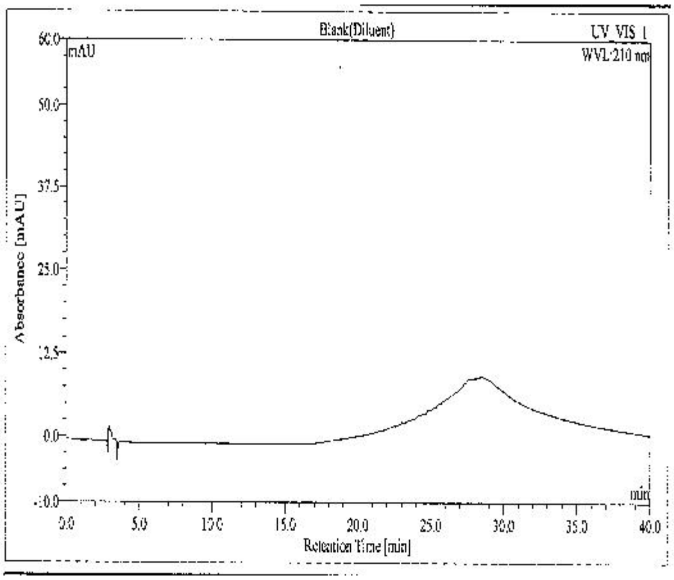

Fig 1. Reference chromatogram of Blank.

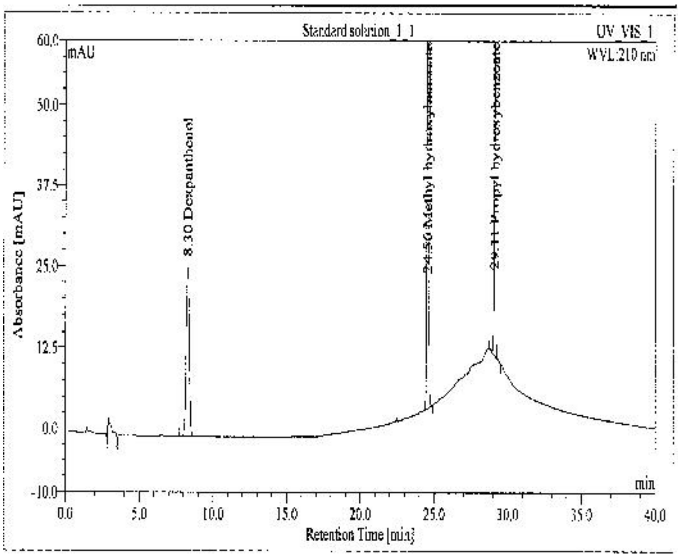

Fig 2. Reference chromatogram of Standard Solution (Dexpanthenol at 210 nm).

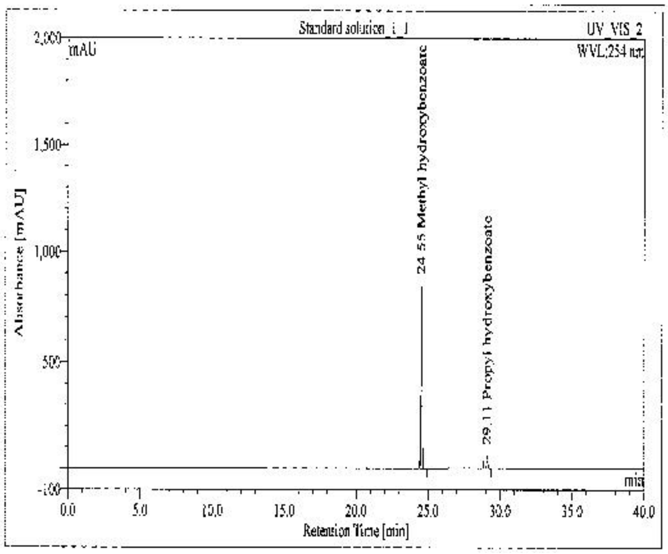

Fig 3. Reference chromatogram of standard solution (Methyl and propyl hydroxy benzoate at 254 nm).

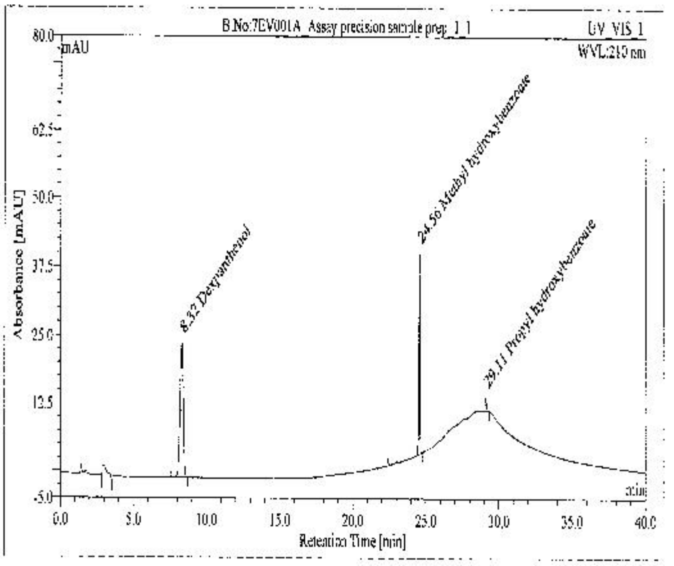

Fig 4. Reference chromatogram of As Such sample (Assay – Dexpanthenol at 210 nm).

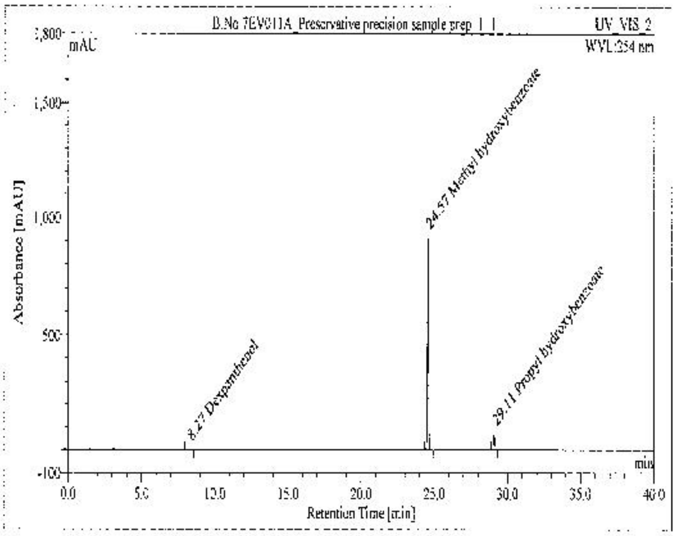

Fig 5. Reference chromatogram of as such sample (Assay–MHB and PHB at 254 nm).

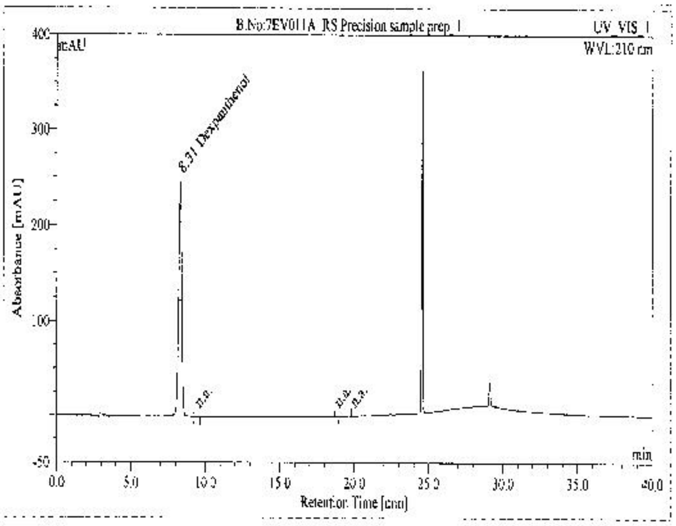

Fig 6. Reference chromatogram of As Such sample (Related substances – Dexpanthenol at 210 nm).

Specificity (Assay, Preservative and RS):

There were no interfering peaks at the retention time of Dexpanthenol, Methyl hydroxybenzoate and Propyl hydroxybenzoate in the presence of excipients. Further, to demonstrate the specificity of the method, the sample had been subjected to acid, base, oxidation, thermal and photolytic degradation. This was evaluated by using a Photo Diode Array detector (PDA). The specificity data is given in Table 5, 6, 6a and 17 for the peak purity analysis data and presented in Fig 7 to 21 for the chromatograms.

Table 5. Forced degradation data of Dexpanthenol.

Sample Name	Area	Assay %	DG %	Purity Match
As such (US)	73064	104.6	-	1.000
AD(@85°C_1h)	1486	2.2	CD	
AD(@85°C_30min	1723	2.5	CD	-
AD(@25°C_20min	48745	71.0	33.6	1.000
BD(@85°C_1h)	0	0.0	CD	-
BD(@85°C_5min)	18623	26.6	78.0	1.000
BD(@25°C_10min	46891	67.9	36.7	1.000
PD(@85 °C_1h)	69222	100.4	4.2	1.000
TD(@105°C_24h)	0	0	CD	-
PhD(1.2Mill. lux h)	69085	96.3	8.3	0.999
Sunlight (24h)	69510	92.6	12.0	1.000

US-Unstressed sample, AD–Acid degradation, CD–Complete degradation, BD–Base degradation, PD–Peroxide degradation, TD–Thermal degradation, DG-Degradation and PhD–Photolytic degradation. Acid and Base: 1M HCl and 1M NaOH. Peroxide: 30% H_2O_2.

Table 6. Forced degradation data of Methyl hydroxybenzoate (MHB).

Sample Name	Area	% Assay	% DG	Purity Match
As such (US) (Unstressed sample)	817189	104	-	0.9998
AD (@85°C_1h)	533466	68.8	35.2	0.9987
BD (@ 85°C_1h)	433	0.1	CD	-
BD (@ 85°C_5min)	21653	2.8	CD	-
BD (@ 25°C_10min)	662078	85.2	18.8	0.9996
PD (@ 85°C_1h)	768239	99.1	4.9	0.9998
TD (@105°C_24h)	741665	94.5	9.5	0.9998
PhD (1.2 Million Lux h)	775570	99.8	4.2	0.9997
Sunlight	744250	91.6	12.4	0.9998

US-Unstressed sample, AD–Acid degradation, CD–Complete degradation, BD–Base degradation, PD–Peroxide degradation, TD–Thermal degradation, DG-Degradation and PhD–Photolytic degradation. Acid and Base: 1M HCl and 1M NaOH. Peroxide: 30% H_2O_2.

Table 6a. Forced degradation data of PHB.

Sample Name	Area	% Assa	% DG	Purity Match
As such (US)	70529	102.9	-	0.9998
AD (@ 85°C_1h)	58011	85.9	17.0	0.9998
BD (@ 85°C_1h)	100	0.1	CD	-
BD (@ 85°C_5min)	19501	28.4	74.5	0.9987
BD (@25°C _10min)	67754	100.0	2.9	0.9997
PD (@ 85°C_1h)	63208	93.5	9.4	0.9998
TD (@105°C_24h)	67476	98.1	4.8	0.9998
PhD (1.2Million lux h)	66923	98.3	4.6	0.9998
Sunlight (24 h)	56715	79.6	23.3	0.9998

US-Unstressed sample, AD–Acid degradation, CD–Complete degradation, BD–Base degradation, PD–Peroxide degradation, TD–Thermal degradation, DG-Degradation and PhD–Photolytic degradation. Acid and Base: 1M HCl and 1M NaOH. Peroxide: 30% H_2O_2.

Table 17. Forced degradation data of Dexpanthenol – Related substances.

Sample Name	Area	Assay %	DG %	Purity Match
As such (US)	703970	100.0	-	0.9996
AD(@85°C_1h)	8898	1.3	CD	0.9977
AD(@25°C_20min	507600	73.4	26.6	0.9999
BD(@85°C_1h)	378	0.1	CD	0.9613
BD(@85°C_5min)	182940	26.0	74.0	1.0000
BD(@25°C_10min	421329	60.5	39.5	1.0000
PD(@85 °C_1h)	416877	60.0	40.0	1.0000
TD(@105°C_24h)	462264	65.9	34.1	0.9999
PhD(1.2Mill. lux h)	589118	84.0	16.0	0.9999
Sunlight DG(24h)	605688	86.3	13.7	0.9999

US-Unstressed sample, A, B, P, T, C DG – Acid, Base, Peroxide, Thermal & Complete degradation. Acid & Base: 1M HCl and 1M NaOH. Peroxide: 30% H_2O_2.

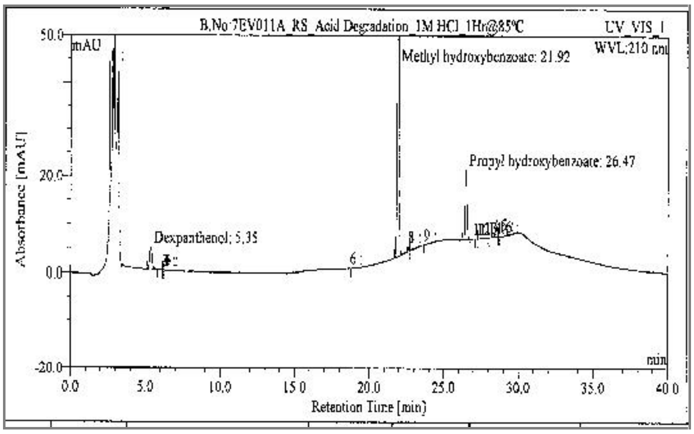

Fig 7. Reference chromatogram of Acid degradation (RS - Dexpanthenol).

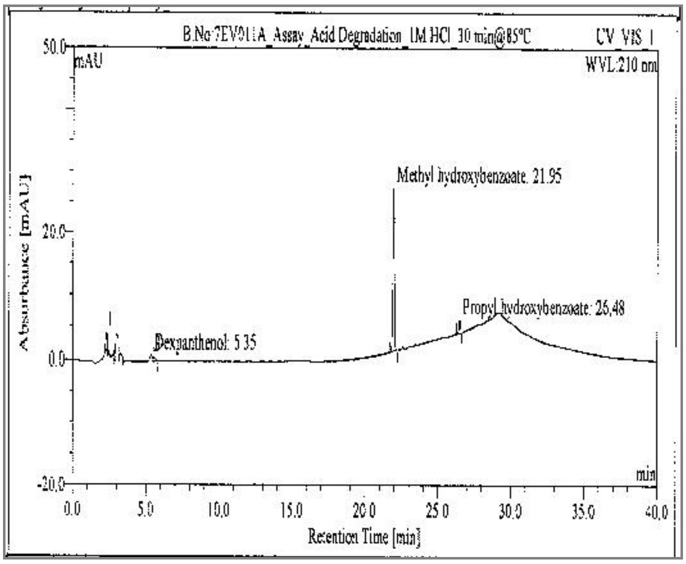

Fig 8. Reference chromatogram of Acid degradation (Assay - Dexpanthenol).

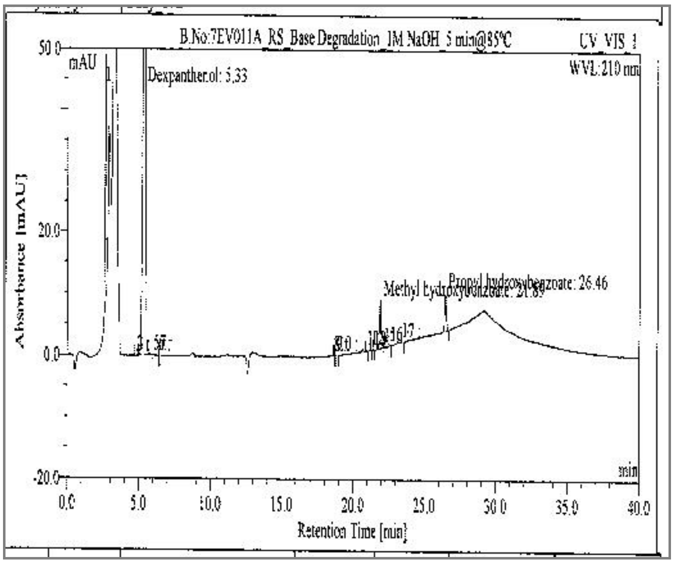

Fig 11. Reference chromatogram of Base degradation (RS - Dexpanthenol).

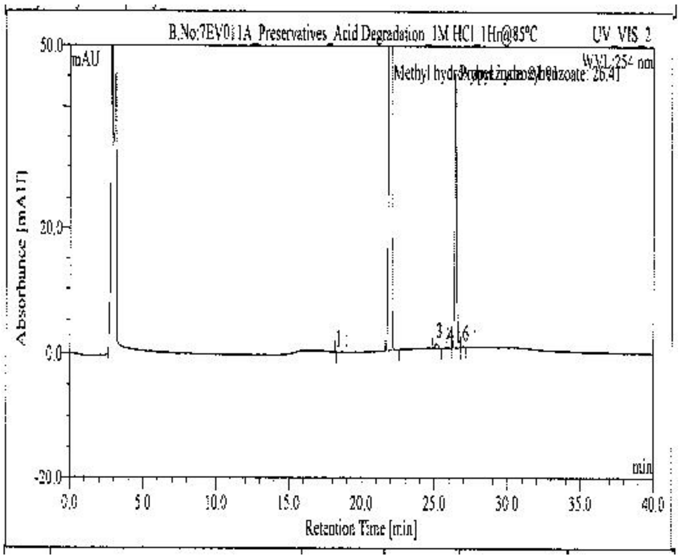

Fig 9. Reference chromatogram of Acid degradation (Preservative).

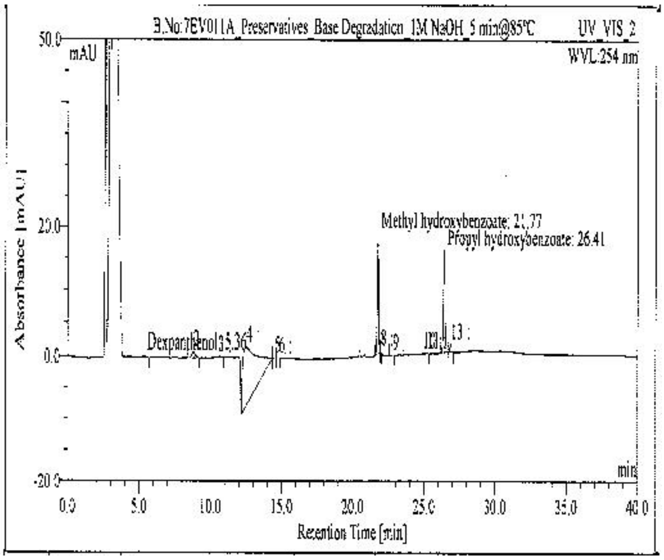

Fig 12. Reference chromatogram of Base degradation (Preservatives).

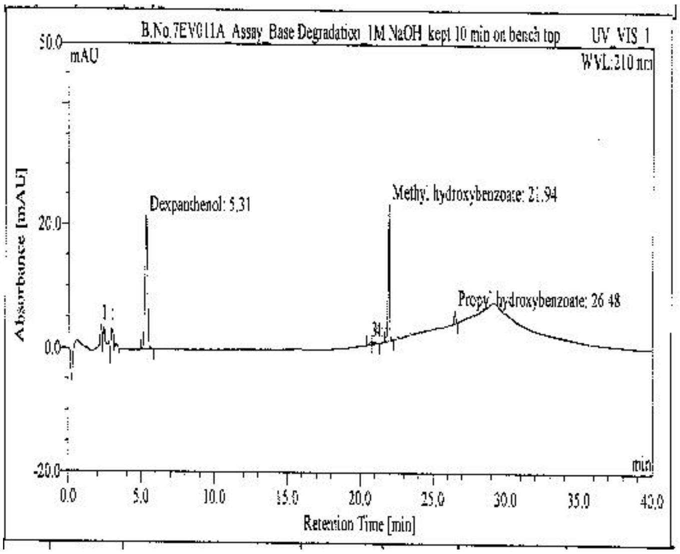

Fig 10. Reference chromatogram of Base degradation (Assay - Dexpanthenol).

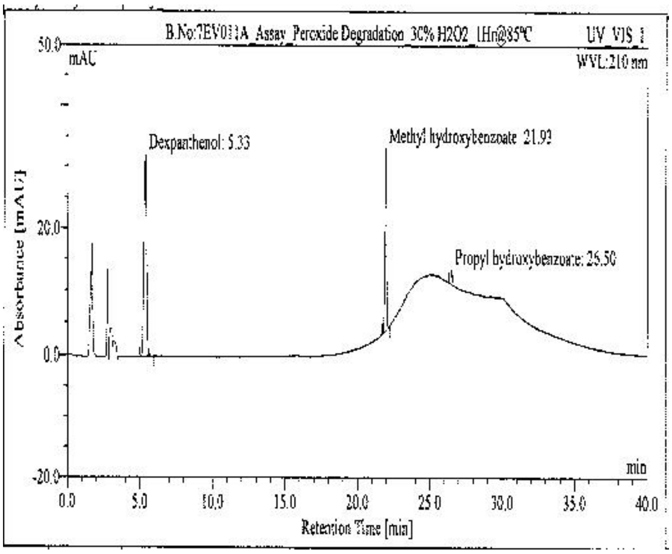

Fig 13. Reference chromatogram of Peroxide degradation (Assay – Dexpanthenol).

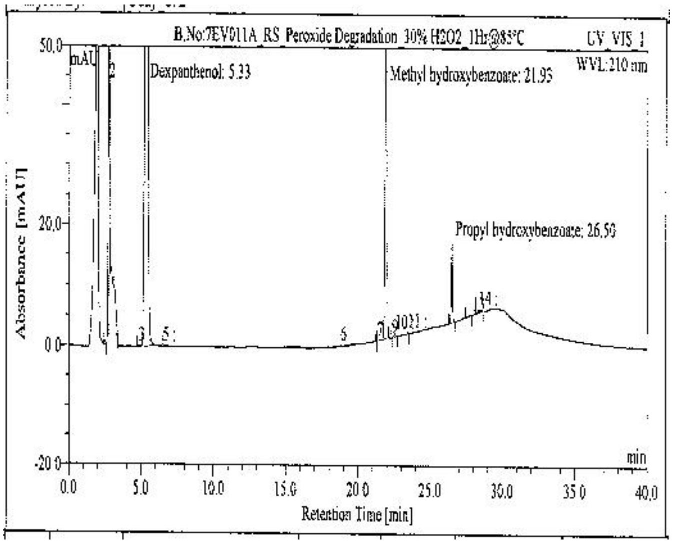

Fig 14. Reference chromatogram of Peroxide degradation (RS – Dexpanthenol).

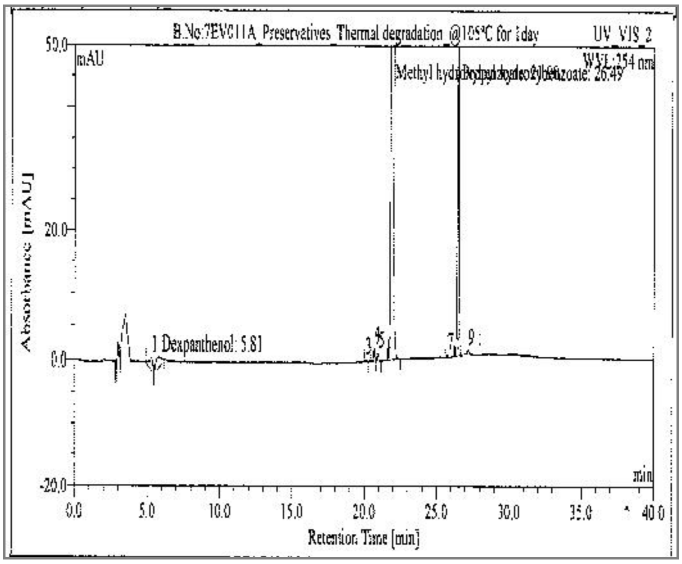

Fig 17. Reference chromatogram of Thermal degradation (Preservatives).

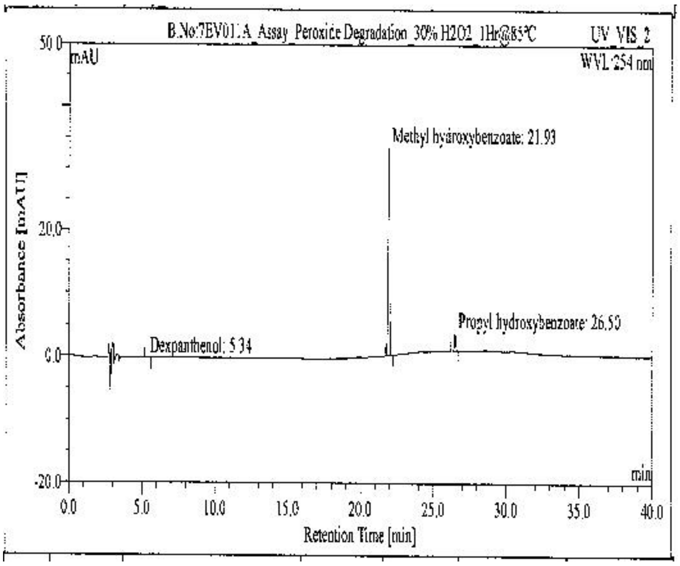

Fig 15. Reference chromatogram of Peroxide degradation (Assay – Dexpanthenol).

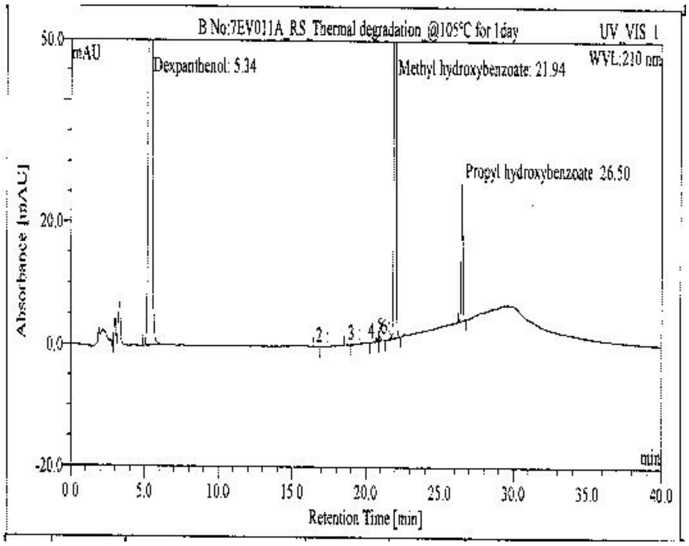

Fig 18. Reference chromatogram of Thermal degradation (RS - Dexpanthenol).

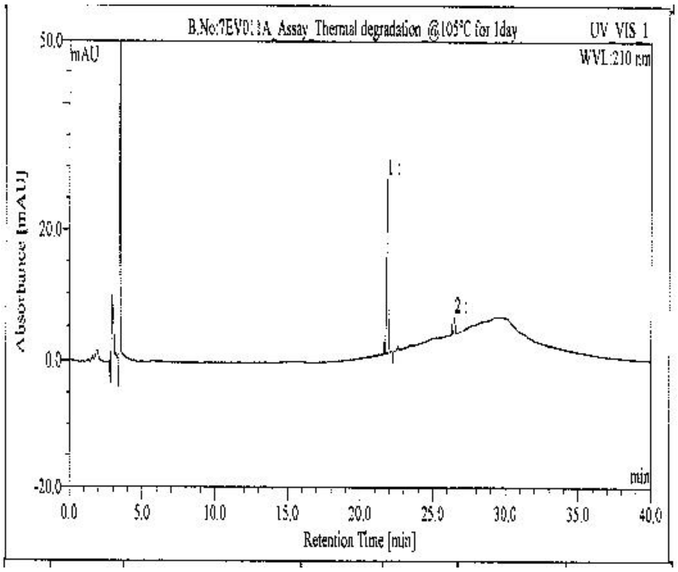

Fig 16. Reference chromatogram of Thermal degradation (Assay - Dexpanthenol).

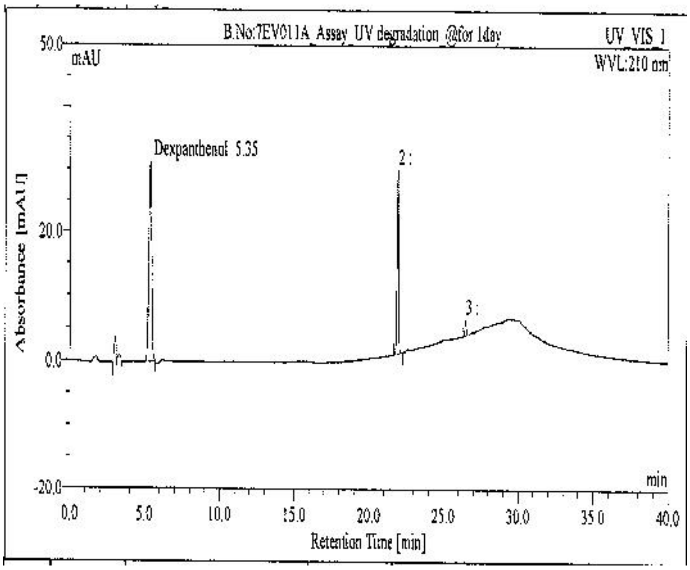

Fig 19. Reference chromatogram of UV degradation (Assay – Dexpanthenol).

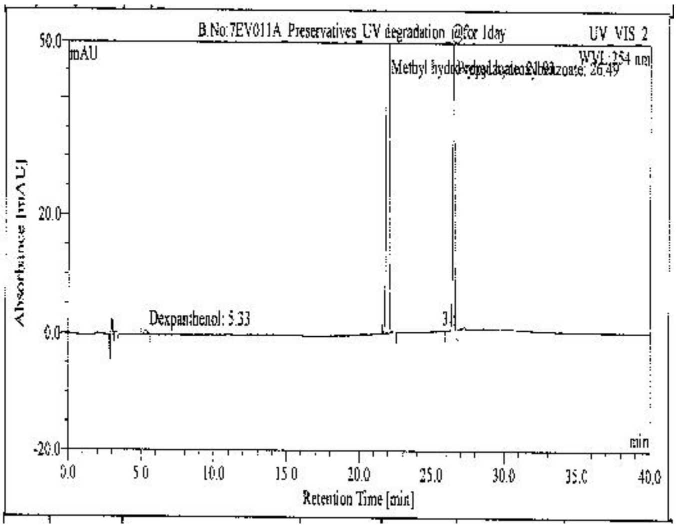

Fig 20. Reference chromatogram of UV degradation (Preservatives).

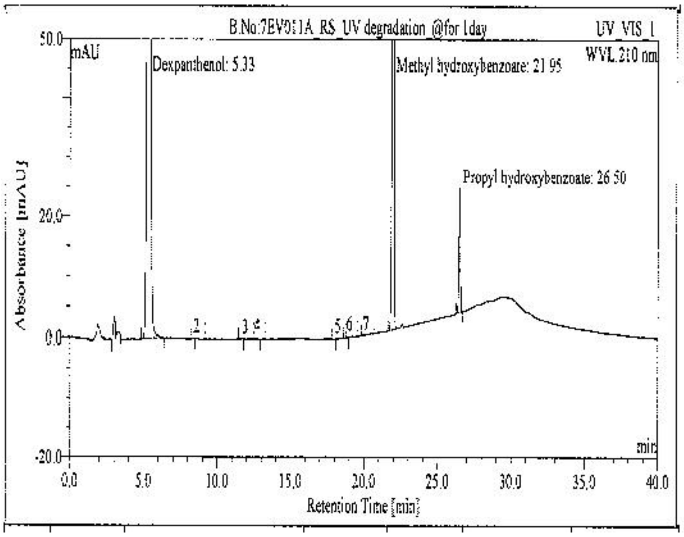

Fig 21. Reference chromatogram of UV degradation (RS – Dexpanthenol).

***Linearity and range (Assay, Preservative and RS)*:**

The standard solutions containing Dexpanthenol, Methyl Hydroxybenzoate and Propyl Hydroxybenzoate were prepared. Linearity was determined by duplicate injections of five different concentrations (50, 80, 100, 120 and 150 % of the target concentration) for Assay and LOQ to 300 % for Related substances). The average peak areas were plotted against concentrations. Then linearity was evaluated using the calibration curve to calculate coefficient of correlation, slope and intercept. In general, a value of correlation coefficient (r^2) > 0.999 is considered as the evidence of an acceptable fit for the data to the regression line.

The results obtained are presented in the Table 10, 11, 12 and 23 which demonstrates that the current method was linear for the three analytes in the range specified above with a correlation coefficients better than 0.999. The plots have been represented in Fig 22 to 25.

Table 10. Linearity data of Dexpanthenol.

Level No.	Concentration (µg/ml)	Mean area
1	25.600	32206
2	40.960	51177
3	46.080	57498
4	51.200	63169
5	56.320	70614
6	61.440	77166
7	76.800	96523
Slope		1258.721
Intercept		-396.071
CC		1.000
R^2		1.000

Table 11. Linearity data of Methyl hydroxybenzoate.

Level No.	Concentration (µg/ml)	Mean area
1	19.998	374699
2	31.996	595324
3	35.996	668429
4	39.995	737095
5	43.995	817979
6	47.994	889685
7	59.993	1114265
Slope		18486.072
Intercept		3140.844
CC		1.000
R^2		1.000

Table 12. Linearity data of Propyl hydroxybenzoate.

Level No.	Concentration (µg/ml)	Mean area
1	1.988	33166
2	3.181	52663
3	3.578	59240
4	3.976	65376
5	4.374	72684
6	4.771	79104
7	5.964	99224
Slope		16625.428
Intercept		-180.273
CC		1.000
R^2		1.000

Table 23. Accuracy of Dexpanthenol for quantification of unknown impurities.

Sl. No.	Level	Sample	Amount recovered (µg/mL)	Amount added (µg/mL)	%Recovery	% RSD in each level
1	LOQ-1	1	0.3280	0.2567	127.8	Avg: 126.7
2	LOQ-2	2	0.3285	0.2567	128.0	SD: 2.081
3	LOQ-3	3	0.3192	0.2567	124.3	%RSD: 1.6
4	50%-1	1	5.5900	5.0330	111.1	Avg: 108.4
5	50%-2	2	5.3679	5.0330	106.7	SD: 2.344
6	50%-3	3	5.4085	5.0330	107.5	% RSD: 2.2
7	100%-1	1	10.7234	10.0660	106.5	Avg: 107.0
8	100%-2	2	10.8204	10.0660	107.5	SD: 0.500
9	100%-3	3	10.7741	10.0660	107.0	% RSD: 0.5
10	150%-1	1	16.1142	15.0990	106.7	Avg: 106.8
11	150%-2	2	16.0547	15.0990	106.3	SD: 0.557
12	150%-3	3	16.2206	15.0990	107.4	% RSD: 0.5

SD – Standard deviation and RSD – Relative Standard deviation.

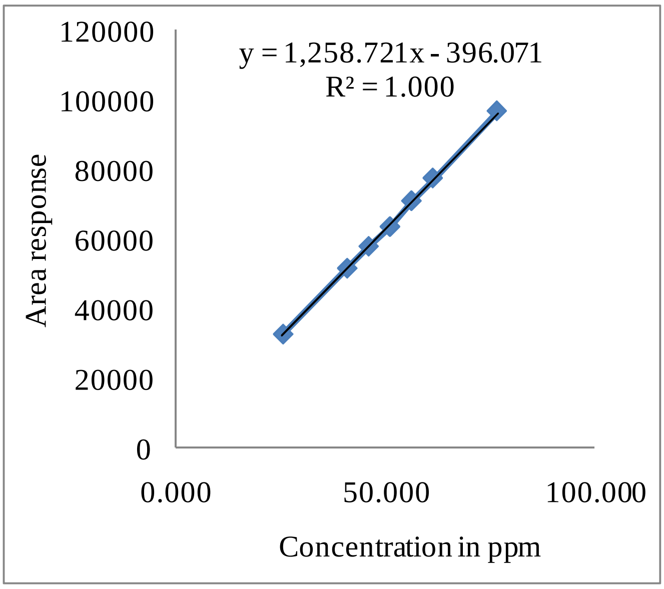

Fig 22. Linearity plot of Dexpanthenol – Assay.

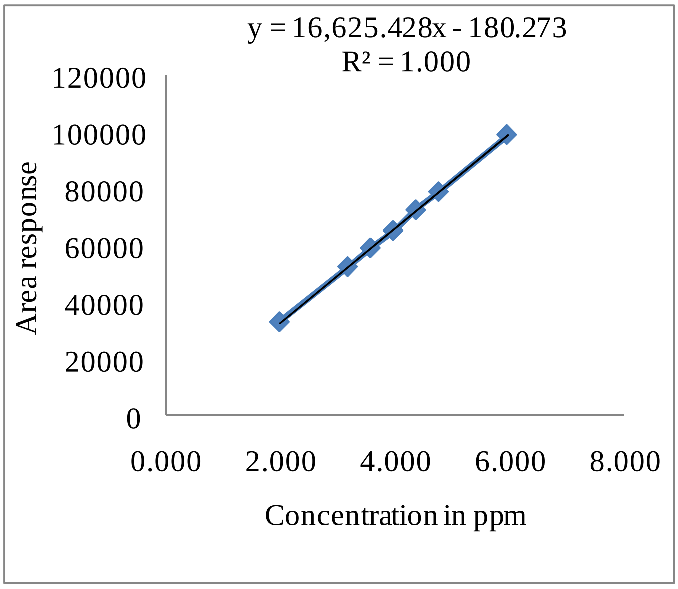

Fig 24. Linearity plot of PHB – Assay.

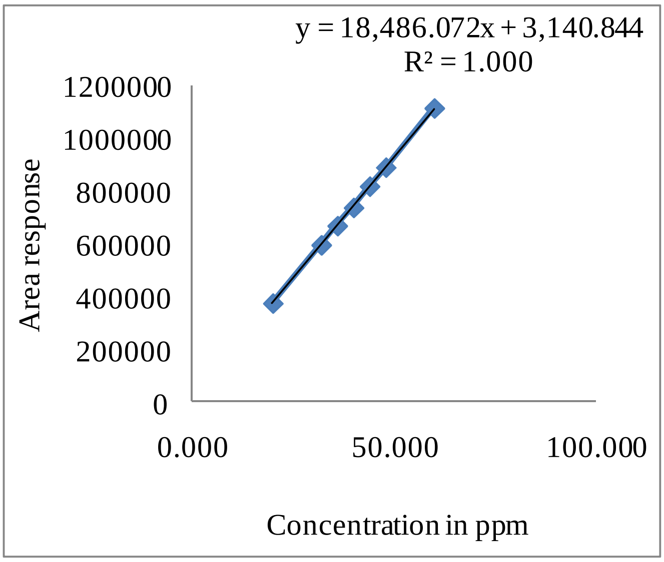

Fig 23. Linearity plot of MHB – Assay.

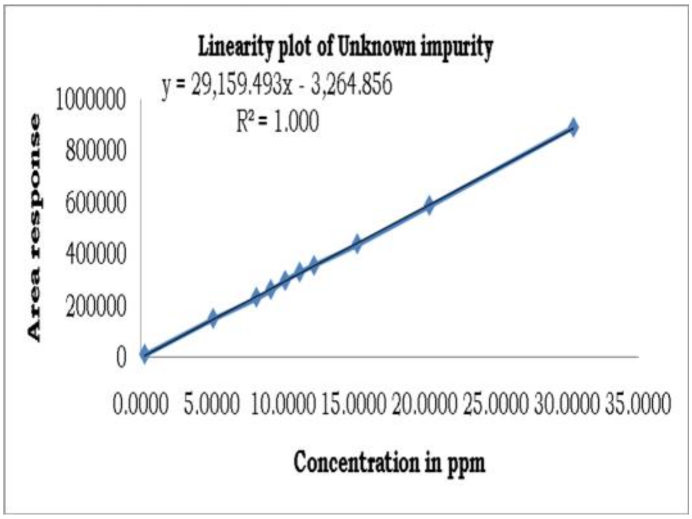

Fig 25. Linearity plot of Dexpanthenol – Related substances.

Precision: (Assay & Preservative & RS)

For Assay, precision was determined by preparing the standard and sample as per the methodology. The sample was prepared in six replicates and injected into the chromatograph. The % Assay value of each preparation was calculated and finally the % RSD of the six replicate preparations was deduced.

Table 7. Precision data of Dexpanthenol, MHB and PHB in Dexanol cream.

Sample ID#	Dexanol cream (B. No: 7EV011A)		
	Dexpanthenol	**MHB**	**PHB**
1	105.1	103.0	101.6
2	104.7	102.7	101.3
3	106.4	103.4	101.9
4	104.6	104.7	103.1
5	104.0	102.5	100.7
6	104.6	103.4	101.9
Mean	104.9	103.3	101.8
SD	0.815	0.783	0.799
% RSD	0.8	0.8	0.8

SD – Standard deviation, RSD – Relative Standard deviation, M&P HB – Methyl & Propyl hydroxybenzoate.

Table 18. Method Precision (% Unknown Impurity) – Related substances.

Sample ID#	Dexpanthenol cream 5 % (B. No: 7EV011A)
1	BQL
2	BQL
3	BQL
4	BQL
5	BQL
6	BQL
Mean	-
SD	-
% RSD	-

SD–Standard deviation, RSD–Relative Standard deviation.

The data obtained for six replicate standard injections and the six sample preparations have been presented in Table 7. For Related substances, precision was established by preparing the sample in six replicates. The data has been presented in Table 18.

Ruggedness: (Assay & Preservative & RS)

Ruggedness of method was demonstrated by preparing the standard and sample as per the methodology by a different analyst on a different day, using a different column lot and using a different HPLC system. The sample was prepared in six replicates and injected into the chromatograph. The % Assay value of each preparation was calculated and finally the % RSD of the six replicate preparations was deduced. The data obtained for six replicate standard injections and the six sample preparations have been presented in Table 8 and 9. For Related substances, intermediate precision was established by preparing the sample in six replicates. The data has been presented in Table 19 to 22.

Table 8. Intermediate Precision data of Dexpanthenol, MHB and PHB in Dexanol cream.

Sample ID#	Dexanol cream (B. No: 7EV011A)		
	Dexpanthenol	**MHB**	**PHB**
1	108.7	103.8	104.4
2	108.6	102.3	101.3
3	108.4	103.4	103.3
4	108.1	103.1	103.1
5	108.0	102.7	102.6
6	107.9	101.6	100.6
Mean	108.3	102.8	102.6
SD	0.331	0.794	1.39
% RSD	0.3	0.8	1.3

SD – Standard deviation, RSD – Relative Standard deviation, MHB and PHB – Methyl and Propyl hydroxybenzoate.

Table 19. Intermediate Precision (% Unknown Impurity) – Dexpanthenol cream.

Sample ID#	Dexpanthenol cream 5 % (B. No: 7EV011A)
1	BQL
2	BQL
3	BQL
4	BQL
5	BQL
6	BQL
Mean	-
SD	-
% RSD	-

SD–Standard deviation, RSD–Relative Standard deviation.

Table 20. Cumulative results of Method Precision and Ruggedness for % Unknown Impurity of Dexpanthenol.

Batch No.	7EV011A	
Preparation ID	**MP**	**IP**
1	BQL	BQL
2	BQL	BQL
3	BQL	BQL
4	BQL	BQL
5	BQL	BQL
6	BQL	BQL
Mean	-	
SD	-	
% RSD	-	

SD–Standard deviation, RSD–Relative Standard deviation.

Table 9. Cumulative % Assay results of Method Precision and Intermediate Precision.

API	Dexpanthenol		Methyl hydroxybenzoate		Propyl hydroxybenzoate	
Preparation ID	**Precision**	**IP**	**Precision**	**IP**	**Precision**	**IP**
1	105.1	108.7	103.0	103.8	101.6	104.4
2	104.7	108.6	102.7	102.3	101.3	101.3
3	106.4	108.4	103.4	103.4	101.9	103.3
4	104.6	108.1	104.7	103.1	103.1	103.1
5	104.0	108.0	102.5	102.7	100.7	102.6
6	104.6	107.9	103.4	101.6	101.9	100.6
Mean	106.6		103.1		102.2	
SD	1.799		0.763		1.119	
% RSD	1.7		0.7		1.1	

IP - Intermediate Precision, SD – Standard deviation and RSD – Relative Standard deviation.

Table 21. Linearity of Dexpanthenol – Related substances.

Level No.	Concentration (µg/ml)	Mean area of Depanthenol
1	0.2536	7963
2	5.0725	146911
3	8.1160	228561
4	9.1305	259180
5	10.1450	293654
6	11.1595	326260
7	12.1740	352218
8	15.2175	436187
9	20.2900	585440
10	30.4350	888252
Slope		29159.493
Intercept		-3264.856
CC		1.000
R^2		1.000

Table 22. Precision at Lower and Higher concentration of Unknown impurity.

Injection No.	Linearity Level-1 (LOQ)	Linearity Level-10 (300 %)
1	7764	868033
2	7859	871042
3	8349	868397
4	7621	865406
5	7581	865611
6	8323	866565
Mean	7916	867509
SD	340.218	2119.498
RSD (%)	4.3 %	0.2 %

SD – Standard deviation and RSD – Relative Standard deviation.

Accuracy: (Assay & Preservative & RS)

For Assay, the accuracy of the proposed method had been demonstrated by the recovery study performed by the standard addition method at levels 50, 100 and 150 % of the target concentration. The data obtained had been presented in Table 13 to 16.

For Related substances, the accuracy of the proposed method had been demonstrated by the recovery study performed by the standard addition method at levels LOQ, 50, 100 and 150 % of the target concentration. The data obtained has been presented in Table 23.

CONCLUSION:

This intended study concludes that the proposed method is economical, simple, sensitive and reliable. Also, it is found to be accurate, precise, specific, stability indicating and rugged. Hence, it can be employed for the routine estimation of Dexpanthenol, its related impurities, Methyl hydroxybenzoate and Propyl hydroxybenzoate in Dexpanthenol topical dosage form.

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REFERENCES:

1. Proksch E, Bony RD, Trapp S, Boudon S. Topical use of dexpanthenol: a 70th anniversary article. J Dermatol Treatment, 2017; 28:8, 766-773.
2. Ebner F, Heller A, Rippke F, Tausch I. Topical Use of Dexpanthenol in Skin Disorders. Am J Clin Dermatol, 2002; 3: 427-433.
3. De AK, Chowdhury PP, Chattapadhyay S. Simultaneous Quantification of Dexpanthenol and

Table 13. Accuracy data of Dexpanthenol.

Sl. No.	Level	Sample	Amount recovered (µg/ml)	Amount added (µg/ml)	Recovery %	% RSD in each level
1	50 %-1	1	25.9668	25.5680	101.6	Avg: 101.3
2	50 %-2	2	25.7182	25.5680	100.6	SD: 0.577
3	50 %-3	3	25.9684	25.5680	101.6	% RSD: 0.6
4	100 %-1	1	51.9691	51.1360	101.6	Avg: 101.3
5	100 %-2	2	51.8056	51.1360	101.3	SD: 0.300
6	100 %-3	3	51.6445	51.1360	101.0	% RSD: 0.3
7	150 %-1	1	75.5586	76.7040	98.5	Avg: 100.1
8	150 %-2	2	77.8674	76.7040	101.5	SD: 1.510
9	150 %-3	3	76.9207	76.7040	100.3	% RSD: 1.5

SD – Standard deviation and RSD – Relative Standard deviation.

Table 14. Accuracy of Methyl Hydroxybenzoate.

Sl. No.	Level	Sample	Amount recovered (µg/ml)	Amount added (µg/ml)	Recovery %	% RSD in each level
1	50 %-1	1	22.0741	21.7000	101.7	Avg: 101.2
2	50 %-2	2	21.9357	21.7000	101.1	SD: 0.503
3	50 %-3	3	21.8593	21.7000	100.7	%RSD: 0.5
4	100 %-1	1	43.6097	43.4000	100.5	Avg: 100.5
5	100 %-2	2	43.1750	43.4000	99.5	SD: 0.950
6	100 %-3	3	44.0200	43.4000	101.4	%RSD: 0.9
7	150 %-1	1	65.1576	65.1000	100.1	Avg: 99.8
8	150 %-2	2	63.9174	65.1000	98.2	SD: 1.429
9	150 %-3	3	65.7214	65.1000	101.0	% RSD: 1.4

SD – Standard deviation and RSD – Relative Standard deviation.

Table 15. Accuracy of Propyl Hydroxybenzoate.

Sl. No.	Level	Sample	Amount recovered (µg/ml)	Amount added (µg/ml)	Recovery %	% RSD in each level
1	50 %-1	1	2.2246	2.1967	101.3	Avg: 100.8
2	50 %-2	2	2.2167	2.1967	100.9	SD: 0.503
3	50 %-3	3	2.2038	2.1967	100.3	%RSD: 0.5
4	100 %-1	1	4.4128	4.3935	100.4	Avg: 100.4
5	100 %-2	2	4.3656	4.3935	99.4	SD: 1.050
6	100 %-3	3	4.4607	4.3935	101.5	%RSD: 1.0
7	150 %-1	1	6.6169	6.5902	100.4	Avg: 100.0
8	150 %-2	2	6.4849	6.5902	98.4	SD: 1.484
9	150 %-3	3	6.6776	6.5902	101.3	% RSD: 1.5

SD – Standard deviation and RSD – Relative Standard deviation.

Table 16. Percentage Assay of Dexpanthenol filtered sample solution with alternative filters.

Formulation details	% Assay of Dexpanthenol (Whatman No.41 filter paper sample)	0.45µm Nylon membrane filter (SNY+GF Grade) (Cat.No:SNY045025H+GF)	0.45µ PVDF	0.45µ PTFE
Dexpanthenol cream 5 % w/w (B.No : 7EV011A)	109.3	107.5	108.0	108.9
% difference		1.8 %	1.3 %	0.4 %

Resorcinol from Hair Care Formulation Using Liquid Chromatography: Method Development and Validation R&D Division. Scientifica, 2016: 2016: 1-8.

4. Doganay A, Koksel B, Gundogdu SO, Capan Y. Simultaneous Determination of Dexpanthenol, Lidocaine Hydrochloride, Mepyramine Maleate and their Related Substances by a RP-HPLC Method in Topical Dosage Forms. J Chromatogr Sci, 2018; 56(10): 903-911.
5. Kelly GS. Pantothenic acid Monograph. Altern Med Rev, 2011; 16: 263-274.
6. Lanska DJ. The discovery of Niacin, Biotin, and Pantothenic acid. Ann Nutr Metab, 2012; 61: 246-253.
7. Williams RJ, Saunders DH. The effects of Inositol, crystalline vitamin B(1) and Pantothenic acid on the growth of different strains of yeast . Biochem J, 1934; 28: 1887-1893.
8. Steuttgen G, Krause H. Percutaneous Absorption of Tritium-Labelled Panthenol in Man and Animal. Arch Klin Exp Dermatol. 1960; 209: 578-582.
9. Abiko Y, Tomikawa M, Shimizu M. Enzymatic conversion of pantothenylalcohol to pantothenic acid. J Vitaminol (Kyoto), 1969; 15: 59-69.
10. Proksch E, Jensen JM, Dexpanthenol. In: Barel AO, Paye M, Maibach HI, editors. Handbook of Cosmetic Science and Technology. 2nd ed. New York, London: Taylor & Francis Group; 2006, pp. 399-406.
11. Proksch E, Holleran WM, Menon GK, *et al*. Barrier function regulates epidermal lipid and DNA synthesis. Br J Dermatol, 1993; 128: 473-482.
12. Slyshenkov VS, Rakowska M, Moiseenok AG, Wojtczak L. Pantothenic acid and its derivatives protect Ehrlich ascites tumor cells against lipid peroxidation. Free Radic Biol Med, 1995; 19: 767-772.
13. Proksch E, Nissen HP. Dexpanthenol enhances skin barrier repair and reduces inflammation after sodium lauryl sulphate-induced irritation. J Dermatolog Treat, 2002; 13: 173-178.
14. Wollina U. The clinical efficacy of dexpanthenol. Kosm Med, 2001; 4: 180-184.
15. Hosemann W, Wigand ME, Geode U, *et al*. Normal wound healing of the paranasal sinuses: clinical and experimental investigations. Eur Arch Otorhinolaryngol, 1991; 248: 390-394.
16. Weimann BI, Hermann D. Studies on wound healing: effects of calcium D-pantothenate on the migration, proliferation and protein synthesis of human dermal fibroblasts in culture. Int J Vitam Nutr Res, 1999; 69: 113-119.
17. Wollina U, Kubicki J. Dexpanthenol supports healing of superficial wounds and injuries. Kosm Med, 2006; 27: 240-249.
18. Marquardt Y, Amann PM, Heise R, *et al*. Characterization of a novel standardized human three-dimensional skin wound healing model using non-sequential fractional ultrapulsed CO_2 laser treatments. Lasers Surg Med, 2015; 47: 257-265.
19. Oguz A, Uslukaya O, *et al*. Topical N-acetylcysteine improves wound healing comparable to dexpanthenol: an experimental study. Int Surg, 2015; 100: 656-561.
20. Danby SG. Biological variation in skin barrier function: from A (atopic dermatitis) to X (xerosis). Curr Probl Dermatol, 2016; 49: 47-60.
21. Proksch E, Bony R, Trapp S, Boudon S. Topical use of dexpanthenol: a 70th anniversary article. J Dermatol Treat, 2017; 28(8): 766-773.

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