Quantitative Estimation of Menthol, Glycerol and 1,2-Propylene Glycol in Tobacco Products Available in India

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Materials: The internal standard *trans*-Anethole, 99% (CAS: 4180-23-8) and 1,3 – Butanediol, 99.5% (CAS: 107-88-0) were purchased from Sigma –Aldrich Co. The standard Menthol (99.9%, analytical standard) were purchased form Supelco, Merck. The standard Glycerol (>99%) and 1,2-Propylene Glycol (>99%) were purchased from Sigma –Aldrich Co. HPLC grade methanol were purchased from Rankem.

Method for the Determination of Menthol[1]:

Sample Preparation for Analysis of Menthol:

For Bidi Samples: Sufficient quantity of bidi tobacco filler were combined and mixed to constitute at least 10 g for each test portion and kept in an airtight amber bottle or container.

For Smokeless Tobacco Samples: Sufficient amount of smokeless tobacco sample sachets were opened and mixed to constitute at least 10 g for each test portion and kept in an airtight amber bottle or container.

Extraction Solvent (500 mg/L): *trans***-**Anethole (99%) was used as an internal standard for the preparation of extraction solvent. 500 mg of Anethole was transferred to 1 L volumetric flask and the volume was made up with methanol.

Preparation of Calibration Standard: The menthol calibration standard were prepared in the range of 20 - 1000 mg/L. At least 5 calibration standards were used for the plotting of calibration curve. If the sample contain higher than the prepared calibration standard then the sample was diluted and the sample was re-analyzed again.

Sample Preparation for Cigarette Samples: Two cigarettes were taken out from the test sample. The cigarettes were slit longitudinally and separated them into cut filler and non-tobacco materials (NTMs; cigarette paper, filter and tipping paper etc.). All materials (cut filler and NTMs) were transferred into the extraction vessel and 40 ml of extraction solvent were pipette out into the extraction vessel. The extraction vessel is placed in an orbital shaker and was shaken for 2 hours. After shaking was completed, the extraction liquid is filtered through 0.22 micron nylon syringe filter and transferred to autosampler amber colored vial and analysed by Gas Chromatograph - FID. If the extract was not analysed on the same day, the solution is stored in a refrigerator.

For the cigarette sample containing menthol ball or capsules, the above procedure was followed with additional steps. The cigarette sample contain menthol ball /capsule in the filter of cigarette and the menthol ball may be broken by pressing the filter with hand without removing the ball / capsule from the filter of cigarette and immediately the filter was transferred to the conical flask.

Sample Preparation for Bidi and Smokeless Tobacco Samples: 1.0 g (weigh 0.001 accuracy) of the tobacco was transferred into the extraction vessel and 40 ml of extraction solvent was pipetted out into the extraction vessel. The extraction vessel was placed in orbital shaker and shaken at 200 RPM for 2 hours. After shaking was completed, the liquid was filtered through 0.22 micron syringe filter and transferred to autosampler amber colored vial and analyzed by Gas Chromatograph - FID. If the extract was not analyzed on the same day, the solution was stored in a refrigerator.

Method for the determination of Glycerol and 1,2-Propylene Glycol[2]:

Sample Preparation for Analysis:

For Cigarette and Bidi Samples: Sufficient quantity of cigarette and bidi tobacco filler were combined and mixed to constitute at least 20 g for each test portion and keep in an airtight amber bottle or container.

For Smokeless Tobacco Samples: Sufficient amount of smokeless tobacco sample sachets were opened and mixed to constitute at least 10 g for each test portion and kept in an airtight amber bottle or container.

Extraction Solution (2000 mg/L):

Primary Stock (200 mg/mL): 5 g 1,3 butanediol was transferred to 25 mL volumetric flask and volume of the flask was made up with methanol.

Preparation of extraction solution (2000 mg/L): 10 mL primary stock was transferred to 1 L volumetric flask and volume of the flask was made up with methanol.

Preparation of Calibration Standard: Glycerol and 1,2-Propylene Glycol calibration standard were prepared in the range of 80 - 3200 mg/L and 30 - 2400 mg/L respectively. At least 5 calibration standards were used for the plotting of calibration curve. If the sample contain higher than the prepared calibration standard then the sample was diluted and the sample was analyzed again.

Sample Preparation for cigarette, and Bidi samples: 4.0 g (weigh 0.001 accuracy) of the sample is taken in 100 ml conical flask and 50 mL of extraction solvent was added using pipette. The solution was shaken at 210 rpm for 60 min. The samples was removed from the shaker and the samples stand undisturbed until the supernatant was clear. The sample solution was filtered through 0.22 micron nylon syringe filter and transferred to an autosampler amber colored vial, and analyzed by GC-FID. (Storage if required: 2-4 °C in dark).

Sample Preparation for smokeless tobacco samples: 2.0 g (weigh 0.001 accuracy) of the sample is taken in 100 ml conical flask and 50 mL of extraction solvent was added using pipette. The solution was shaken at 210 rpm for 60 min. The samples was removed from the shaker and the samples stand undisturbed until the supernatant was clear. The sample solution was filtered through 0.22 micron nylon syringe filter and transferred to an autosampler amber colored vial, and analyzed by GC-FID. (Storage if required: 2-4 °C in dark).

Table 1: **Determination of** menthol content in 35 brands of zarda / chewing tobacco (SLT) samples.

Sl. No.	Brand name	Menthol (mg/g)	Sl. No.	Brand name	Menthol (mg/g)
1	Ratna 64	1.67 (0.03)	19	Shivdata 355	9.19 (0.15)
2	Kanak Deluxe	2.24 (0.23)	20	Balaji-128 deluxe	9.25 (0.04)
3	PP	3.36 (1.05)	21	Baba -120	8.70 (1.22)
4	Baba Black	3.90 (0.40)	22	Mastana 142	9.45 (0.06)

Sl. No.	Brand name	Menthol (mg/g)	Sl. No.	Brand name	Menthol (mg/g)
5	SNT -1	4.35 (0.62)	23	MJ Zarda Patti	10.22 (0.17)
6	Suravi 55	4.57 (0.18)	24	Krishna 60	10.27 (0.08)
7	Gopal Green	4.97 (0.16)	25	Baba-160	11.46 (0.30)
8	V-1	5.69 (0.81)	26	KP Double Black	13.72 (1.27)
9	BHR	6.64 (0.63)	27	Sigma Tobacco	13.78 (0.16)
10	Sourabi 58	6.80 (0.26)	28	Parijat Tobacco	13.9 (0.31)
11	SS-1	7.18 (0.71)	29	Green label	14.18 (1.28)
12	KC 2000	7.42 (0.27)	30	Gopal - 132	14.39 (0.98)
13	Action 7	7.52 (0.17)	31	Silver class-2	14.01 (0.92)
14	Gopal 60	7.89 (0.59)	32	Classic Zafrani Zarda	19.84 (0.24)
15	Baba 54	8.12 (0.06)	33	Ashirbad	21.13 (0.28)
16	Tulsi	8.56 (0.27)	34	Ratna Tobacco 300	21.81 (0.22)
17	Sonali 155	8.82 (0.27)	35	UK tobacco No. 3300	26.99 (0.46)
18	M-4	8.85 (0.34)			

The samples were analyzed and the mean value was presented. Standard deviation (SD) was shown in parenthesis.

Table 2: Menthol content in different brands of smokeless tobacco samples (Gul, Gurakhu, Snuff, Snus, Sada, Khaini and Kiwam).

Sl. No.	Brand Name	Types of SLT	Mean Menthol (mg/g)	Sl. No.	Brand Name	Types of SLT	Mean Menthol (mg/g)
1	AQ Premium Kara Gul	Gul	ND	23	Singham Khaini	Khaini	ND
2	Chand tara marka special gul		ND	24	Chandan Khaini		ND
3	Dulhan Gul		ND	25	Dada Khaini		ND
4	Gulab marka Gul		ND	26	Nevla Tobacco		ND
5	Kale Gulab marka Gul		ND	27	New Lucky Khaini		ND
6	Khan Ka Gul		ND	28	Rakesh Khaini		ND
7	Kohinoor Gul		ND	29	Arif Khaini		0.32 (0.01)
8	Noor Ka Gul		ND	30	Swagat No. 1		1.5 (0.01)
9	S. S. Special Kara Gul		ND	31	Afgan Rally		2.8 (0.01)
10	Sameer Ka Gul		ND	32	Dilkhus khaini Spitt tobacco		3.5 (0.03)

Sl. No.	Brand Name	Types of SLT	Mean Menthol (mg/g)	Sl. No.	Brand Name	Types of SLT	Mean Menthol (mg/g)
11	Annapurna Gurakhu		ND	33	Saajan Khaini		4.0 (0.11)
12	Trishul Gurakhu		ND	34	Bandar Brand XXX		4.6 (0.03)
13	Tobacco Gurakhu		ND	35	Chunnu Right Choice		6.0 (0.06)
14	Tobacco Gurakhu	Gurakhu	ND	36	Raja Tobacco		8.7 (1.24)
15	Parbati kara Gurakhu		ND	37	Golden Sada		3.5 (0.16)
16	Tarakeshwar Gurakhu		ND	38	Suraj Chaap Tambaku		0.23 (0.02)
17	Shiv Shakti Gurakhu		ND	39	Banadar Sada (Paper Packet)	Sada	ND
18	N. C. Madras Snuff		ND	40	J.A. P.D.M Patta Sada		ND
19	N. K. Madras Snuff	Snuff	ND	41	Joyguru Sada		ND
20	No 1 NC Gold Snuff		ND	42	Kaka sada		0.34 (0.11)
21	Chaini Tobacco	Pouch Khaini / Snus	7.6 (0.5)	43	Tulsi Royal Special Kiwam	Kiwam	24.9 (1.15)
22	Bolt Filter		8.3 (0.3)	44	Raj Ratan tobacco		13.0 (0.18)

The samples were analyzed and the mean value was presented. Standard deviation (SD) was shown in parenthesis. ND: Not detected in the sample. Limit of Detection = 0.014 mg/g.

Table 3: Determination of Menthol content in different brands of cigarette samples.

Sl. No.	Brand Name	Menthol (mg/Cig.)
1	Silk Cut	0.02 (0.001)
2	Gold flake Premium Honey Dew Blend	0.07 (0.02)
3	Gold Flake Indie Mint	2.28 (0.04)
4	Total	3.28 (0.79)
5	Wave Cool Mint	3.88 (0.14)
6	Wave	3.86 (0.01)
7	Wave Fruit Mint	5.14 (0.01)
8	Players	5.20 (0.26)
9	Topaz	6.40 (0.22)
10	Classic	7.38 (0.06)

Sl. No.	Brand Name	Menthol (mg/Cig.)
11	Marlboro	9.27 (0.05)
12	Flake	ND
13	Classic Refined Taste	ND
14	Classic Balanced Taste	ND
15	Wills Navy Cut	ND
16	Cavanders Gold	ND
17	Gold Flake	ND
18	P-10 premium	ND
19	Charms	ND
20	U -10	ND
21	Gold Flake Kings Honey dew Smooth	ND
22	Four Square Special Advanced Taste	ND
23	H-10 Filter	ND
24	Esse Lights	ND
25	Sunils Filter	ND
26	WIN	ND
27	Djarum Black	ND
28	Scissors	ND

The samples were analyzed and the mean value was presented. Standard deviation (SD) was shown in parenthesis.

Table 4: Determination of Menthol content in different brands of Bidi Samples.

Sl. No.	Brand Name	Menthol (mg/g)
1	Royal Tara Biri	ND
2	Sagar Bidi	ND
3	Pal Bidi	0.15 (0.01)
4	Nandini Bidi	<LOQ
5	Lallu bidi	ND
6	No. 1 mantu bidi	ND
7	Janata Biri	ND
8	Mother India Biri	<LOQ
9	Sankha Biri	0.21 (0.001)
10	Jadu Biri	ND
11	Pataka Bidi	ND
12	Deep Bidi	ND
13	Saheb Bidi	ND

Sl. No.	Brand Name	
14	Sushantor Bidi	ND
15	Mondoler Bidi	ND

The samples were analyzed and the mean value was presented. Standard deviation (SD) was shown in parenthesis. Limit of Detection = 0.014 mg/g. Limit of Quantitation (LOQ) = 0.042 mg/g.

Table 5: Determination of Additives (Glycerol and 1,2-Propylene Glycol) in 35 different representative brands of chewing tobacco and zarda samples:

Sl. No.	Brand Name	Humectant	
		Glycerol (mg/g)	**1,2-Propylene glycol (mg/g)**
		Range: 0.9 – 185.3	**Range: ND – 40.8**
1	Baba 120	0.93 (0.07)	1.48 (0.06)
2	Baba 54	0.97 (0.13)	1.05 (0.02)
3	Sonali 155	1.1 (0.01)	ND
4	Tulsi	1.2 (0.01)	4.9 (4.5)
5	Baba 160	1.4 (0.01)	1.4 (0.14)
6	Sourabi 58	1.5 (0.06)	ND
7	Shivdata 355	2.2 (0.02)	ND
8	MJ Zarda Patti	3.2 (0.4)	0.6 (0.03)
9	BHR	4.4 (0.4)	4.6 (0.46)
10	SNT-1	9.3 (1.2)	ND
11	V-1	20.5 (2.8)	ND
12	Action 7	21.8 (0.4)	1.7 (0.4)
13	KP Double black	23.8 (2.4)	23.7 (3.5)
14	KP Silver class-2	24.4 (0.8)	27.5 (0.6)
15	KC 2000	26.5 (2.6)	40.8 (1.1)
16	Green Label	27.3 (3.0)	28.2 (3.2)
17	Gopal Green	28.5 (4.4)	<LOQ
18	Mastana 142	28.7 (0.05)	1.6 (0.01)
19	M-4	37.1 (4.3)	18.1 (0.04)
20	UK tobacco No. 3300	39.1 (0.6)	1.4 (0.01)
21	SS-1	39.7 (2.3)	<LOQ
22	Gopal 132	42.2 (1.3)	3.3 (0.16)
23	Suravi 55	45.0 (0.6)	ND
24	Gopal 60	45.9 (3.9)	3.6 (0.27)
25	Parijat Tobacco	54.2 (0.24)	ND
26	Ashirbad	55.4 (1.4)	11.8 (0.13)

Sl. No.	Brand Name	Humectant	
		Glycerol (mg/g)	1,2-Propylene glycol (mg/g)
27	Balaji-128 deluxe	57.6 (0.9)	ND
28	Kanak Deluxe	65.7 (1.80)	ND
29	Sigma Tobacco	78.7 (2.3)	ND
30	Baba Black	83.7 (0.5)	ND
31	PP	86.2 (0.2)	4.0 (0.01)
32	Ratna 64	114.5 (0.7)	ND
33	Classic Zafrani Zarda	168.2 (1.2)	1.3 (0.01)
34	Krishna 60	177.6 (2.8)	<LOQ
35	Ratna No. 300	185.3 (7.8)	ND

The samples were analyzed and the mean value was presented. Standard deviation (SD) was shown in parenthesis. ND: Below Detection limit. Limit of Detection (LOD) of Glycerol = 0.3 mg/g and 1,2-Propylene Glycol = 0.06 mg/g; Limit of Quantitation of Glycerol = 0.9 mg/g and 1,2-Propylene Glycol = 0.2 mg/g.

Table 6: Glycerol and 1,2-Propylene Glycol content in smokeless tobacco samples such as gul, gurakhu, snuff, sada and khaini products.

Sl. No.	Brand name	Types of SLT	Glycerol (mg/g)	1,2-Propylene Glycol (mg/g)
1	AQ Premium Kara Gul		ND	ND
2	Chand tara marka special gul		ND	ND
3	Dulhan Gul		ND	ND
4	Gulab marka Gul		ND	ND
5	Kale Gulab marka Gul	Gul	ND	ND
6	Khan Ka Gul		ND	ND
7	Kohinoor Gul		ND	ND
8	Noor Ka Gul		ND	ND
9	S. S. Special Kara Gul		ND	1.11 (0.06)
10	Sameer Ka Gul		1.12 (0.08)	ND
11	Annapurna Gurakhu		ND	ND
12	Trishul Gurakhu		ND	ND
13	Tobacco Gurakhu	Gurakhu	ND	ND
14	Tobacco Gurakhu		ND	ND
15	Parbati kara Gurakhu		ND	ND

Sl. No.	Brand name	Types of SLT	Glycerol (mg/g)	1,2-Propylene Glycol (mg/g)
16	Tarakeshwar Gurakhu		ND	ND
17	Shiv Shakti Gurakhu		ND	ND
18	N. C. Madras Snuff	Snuff	ND	ND
19	N. K. Madras Snuff		ND	ND
20	No 1 NC Gold Snuff		ND	ND
21	Chaini Tobacco	Pouch khaini / Snus	53.4 (3.2)	2.1 (0.01)
22	Bolt Filter		39.52 (0.12)	16.62 (0.05)
23	Arif Khaini		ND	ND
24	Chandan Khaini		ND	ND
25	Dada Khaini		ND	ND
26	Nevla Tobacco	khaini	<LOQ	ND
27	New Lucky Khaini		ND	ND
28	Rakesh Khaini		ND	ND
29	Singham Khaini		ND	ND
30	Swagat No. 1		4.56 (0.01)	ND
31	Afgan Rally		1.23 (0.06)	ND
32	Dilkhus khaini Spitt tobacco		2.50 (0.22)	ND
33	Saajan Khaini	khaini	ND	ND
34	Bandar Brand XXX		<LOQ	ND
35	Chunnu		<LOQ	ND
36	Raja Tobacco		<LOQ	0.64 (0.03)
37	Golden Sada		ND	ND
38	Suraj Chaap Tambaku		ND	ND
39	Banadar Sada (Paper Packet)	Sada	ND	ND
40	J.A. P.D.M Patta Sada		ND	ND
41	Joyguru Sada		ND	ND
42	Kaka sada		ND	ND
43	Tulsi Royal Special Kiwam	Kiwam	2.3 (0.05)	23.9 (1.3)
44	Raj Ratan tobacco		456.3 (17.6)	ND

The samples were analyzed and the mean value was presented. Standard deviation (SD) was shown in parenthesis. ND: Not detected in the sample. Limit of Detection (LOD) of Glycerol = 0.3 mg/g and 1,2-Propylene Glycol = 0.06 mg/g; Limit of Quantitation of Glycerol = 0.9 mg/g and 1,2-Propylene Glycol = 0.2 mg/g

Table 7: Determination of additives (glycerol and 1,2-propylene glycol) in different brands of cigarette samples (Smoke Tobacco):

Sl. No.	Brand Name	Glycerol (mg/g)	1,2-Propylene Glycol PG (mg/g)
1	P-10 premium	ND	ND
2	Charms	ND	0.9 (0.01)
3	H-10 Filter	ND	<LOQ
4	Sunils Filter	ND	<LOQ
5	U -10	1.06 (0.04)	ND
6	Djarum Black	2.16 (0.08)	10.70 (0.4)
7	Gold Flake Kings Honey dew Smooth	2.17 (0.6)	<LOQ
8	Topaz	4.39 (0.07)	3.02 (0.10)
9	Four Square Special Advanced Taste	5.40 (0.13)	3.89 (0.10)
10	Wave Cool Mint	5.7 (0.23)	0.5 (0.01)
11	Total	6.4 (0.4)	1.0 (0.13)
12	Cavanders Gold	7.5 (0.34)	2.5 (0.22)
13	WIN	7.90 (0.01)	4.96 (0.28)
14	Classic	8.21 (0.03)	3.33 (0.01)
15	Classic Refined Taste	9.5 (0.14)	<LOQ
16	Scissors	9.60 (0.08)	ND
17	Flake	11.2 (1.3)	<LOQ
18	Wave Fruit Mint	10.12 (0.4)	0.33 (0.01)
19	Gold Flake	10.2 (0.9)	<LOQ
20	Wills Navy Cut	12.6 (1.3)	<LOQ
21	Wave	12.2 (0.5)	0.3 (0.01)
22	Players	12.6 (1.3)	<LOQ
23	Silk Cut	14.2 (0.04)	0.6 (0.04)
24	Gold Flake Indie Mint	14.95 (0.20)	0.6 (0.01)
25	Gold Flake Premium Honey Dew Blend	18.0 (1.4)	0.7 (0.1)

Sl. No.	Brand Name	Glycerol (mg/g)	1,2-Propylene Glycol PG (mg/g)
26	Esse Lights	17.71 (0.44)	4.96 (0.3)
27	Classic balanced Taste	21.03 (0.4)	1.05 (0.01)
28	Marlboro	22.33 (0.4)	2.18 (0.01)

The samples were analyzed and the mean value was presented. Standard deviation (SD) was shown in parenthesis. LOQ of Glycerol = 0.9 mg/g and LOQ of 1,2-Propylene glycol = 0.2 mg/g).

Table 8: Determination of Glycerol and 1,2-Propylene Glycol content in different brands of Bidi Samples (smoke).

Sl. No.	Brand Name	Glycerol (mg/g)	1,2-Propylene Glycol (mg/g)
1	Royal Tara Biri	ND	ND
2	Lallu bidi	ND	ND
3	Mother India Biri	ND	ND
4	Sankha Biri	ND	ND
5	Janata Biri	ND	ND
6	Jadu Biri	ND	ND
7	Mondoler Bidi	ND	ND
8	Sagar Bidi	1.1 (0.07)	ND
9	Nandini Bidi	1.41 (0.01)	ND
10	No. 1 mantu bidi	1.42 (0.01)	ND
11	Pal Bidi	1.71 (0.01)	ND
12	Sushantor Bidi	1.72 (0.01)	ND
13	Saheb Bidi	1.81 (0.01)	ND
14	Pataka Bidi	2.06 (0.01)	ND
15	Deep Bidi	3.63 (0.03)	ND

The samples were analyzed and the mean value was presented. Standard deviation (SD) was shown in parenthesis. ND: Not detected in the sample. Limit of Detection (LOD) of Glycerol = 0.3 mg/g and 1,2-Propylene Glycol = 0.06 mg/g; Limit of Quantitation of Glycerol = 0.9 mg/g and 1,2-Propylene Glycol = 0.2 mg/g

References:

1. Determination of Menthol in Cigarettes and Cut Filler by Gas Chromatography. CORESTA Recommended Method No. 92 (2019).

2. Standard Operating Procedure for the Determination of Humectants in Cigarette Tobacco Filler. WHO TobLabNet Official Method SOP 06 (2016).