

Analytical study and HPTLC profile of Panchagavya

Ghrit- An Ayurvedic medicine

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Abstract: Panchagavya Ghri, an Ayurvedic medicine has been indicated in Ayurvedic classics in Apasmara (Epilepsy), Kamala (Jaundice), Jwara (Fever) (1), for Pandu (Anaemia) (2) and Vishamajwara (Malaria) (3). Its use in the treatment of malignant situations is also a popular practise. Various physico-chemical characteristics, qualitative tests, and HPTLC were used to construct the technique for its analysis. For its quality control, the evolving data and HPTLC profile would be quite helpful.

Keywords: Panchagavya Ghrit, Physico-chemical, Quality control, HPTLC, Standardization

INTRODUCTION:

Panchagavya Ghri, an Ayurvedic medicine has been indicated in Ayurvedic classics in Apasmara (Epilepsy), Kamala (Jaundice), Jwara (Fever), for Pandu (Anaemia) and Vishamajwara (Malaria). Recently, its use in the treatment of malignant situations is also a popular practise. The use of Panchagavya Ghrit has been recommended for the neurogenic and psychogenic illnesses (4,5).

Any drug's quality must be maintained. Physico-chemical characteristics, qualitative and quantitative tests and chromatographic studies are a few examples of the various analytical techniques that can be used to conduct it. Particularly for Ayurvedic products, which contain a variety of chemical moieties and a very little is known about the therapeutically active compound, physico-chemical parameters and preliminary phytochemicals analysis play a crucial role in analysis. They aid in the standardisation, quality assurance, and authentication of Ayurvedic products. There is no any data regarding analytical study of Panchagavya Ghrit in our literature. Hence the analytical study of Panchagavya Ghrit was undertaken.

MATERIALS AND METHODS

Solvents and Chemicals: All solvents and chemicals used were of analytical grade from Merck India, Mumbai.

Preparation of Panchagavya Ghrit: PGG was prepared in our laboratory by following the classical method described in *Charaka Samhita* (6). The raw materials namely dung, urine and milk were obtained from Gir variety of indigenous cow, that is *Bos indicus*, reared in house in the Gaushala run by Shree Prabhav Hem KamdhenuGirivihar Trust, Wagaldhara, Valsad, Gujarat. The curd and Ghrit were prepared by processing milk from the same source.

Five hundred grams of freshly collected cow dung was mixed homogeneously with 500 mL of water and the mixture was strained through a cloth. This dung extract (about 500 mL), along with 500 mL each of freshly collected cow urine, cow milk (boiled and cooled), and curd from cow milk (night old) were mixed with 500 mL of molten cow ghee and heated on mild heat with continuous stirring. The process was continued uninterruptedly for about 3-3.5 h till separation of phases occurred and then allowed to cool. After keeping it overnight, next day, the processing was continued on mild heat till *Siddhi Lakshanas* (7) (~ process termination signs) were obtained.

Preparation of Panchgavya

In the early morning fresh cow product i.e. Milk, Dung and urine was collected and indirect product Ghee and Curd were also collected freshly in equal proportion. After that Cow Urine and dung was thoroughly mixed and filtered eight times by using fine cotton cloth followed by mixing of milk and curd in filtrate and again filtered it by using cotton cloth.

In the end the filtered was boiled until removal of water and ghee was added in the end to prepared panchgavyaghrita. Sample analysis was carried out by using various parameters and the evolved data was analysed.

A. Physico-Chemical Parameters: Various physico-chemical parameters like specific gravity, residue on evaporation, acid value, saponification value, unsaponifiable matter and qualitative tests were employed. The specific gravity was determined at room temperature by using specific gravity bottle; the acid value, saponification value and unsaponifiable matter were determined as described in I.P (8,9). Determination of residue on evaporation – About 10 g, accurately weighed, sample was taken in a previously dried and weighed evaporating dish. It was heated on a water bath to obtain a residue (ROE). The weight of the residue was noted and expressed in terms of %w/w.

1. **Qualitative Tests:** A portion of ROE was extracted with methanol and the methanol extractwas tested qualitatively (10) for the presence of different types of compounds.

Samples:

- I. 2 g sample was adsorbed with 2 g silica gel G for TLC powder, dried at room temperature and then extracted successively with petroleum ether ($60^{0}-80^{0}$ C) and ethyl acetate. After suitable dilution the spectra were recorded (400-800 nm for petroleum ether and 200-300 nm for ethyl acetate extract).
- II. The unsaponifiable matter of the sample was dissolved in ether and the spectrum (190-1100 nm) was recorded.

B. HPTLC profile: TLC / HPTLC profile of natural products is very useful for detecting batch to batch variation and is widely used for analysis of plant based products as well as other products of natural origin. So, an attempt was made to develop HPTLC profile of Panchagavya Ghrit.

Chromatographic Conditions:

Sample preparation: A portion of ROE was extracted successively with petroleum ether and methanol. A part of methanol extract was kept as such and the remaining part was evaporated to dryness. The residue was extracted successively with chloroform, ethyl acetate and methanol. All the extracts viz. petroleum ether extract (PE), methanol extract (ME), chloroform (CH), ethyl acetate (EA) and methanol (MES) fractions were used for application. HPTLC was performed on 10 cm x 10 cm aluminum plates coated with 0.2 mm layer of silica gel $60F_{254}$ (Merck, Germany). Sample was applied in band with a Linomat V applicator CAMAG (Switzerland), equipped with a $100-\mu$ l syringe. Plates were developed vertically, in a CAMAG twin trough chamber previously saturated with mobile phase vapour for 20 min at room temperature. Various mobile phases were tried and finally the following systems were used for the present study. For PE: Hexane - Diethyl ether - Glacial acetic acid (65:35:2) for other fractions: Toluene - Ethyl acetate - Glacial acetic acid (6.5:3.5:0.2). Detection was carried out under UV and spraying with 5% ferric chloride solution as well as anisaldehyde sulphuric acid reagent followed by heating at 110^{0} C for 5-10 minutes under observation. Anisaldehyde-sulphuric acid reagent was prepared by mixing 5 ml anisaldehyde with 50 ml glacial acetic acid and 1 ml concentrated sulphuric acid, in that order (11).

RESULTS AND DISCUSSION:

Physico-chemical parameters and Qualitative test: The data of physico-chemical parameters of the samples has been presented in Table-1A general screening was conducted by performing various qualitative tests to have an idea about the type of chemical constituents present in Panchagavya Ghrit. The results revealed the presence of secondary metabolites like phenolic compounds, steroid/triterpenes and flavonoids.

HPTLC study: HPTLC study - For optimization of mobile phase, resolution and number of spots

Analytical study and HPTLC profile of Panchagavya Ghrit– An Ayurvedic medicine

were taken into consideration. Among the different mobile phases tried, Hexane - Diethyl ether - Glacial acetic acid (65:35:2) for PE and Toluene - Ethyl acetate - Glacial acetic acid (6.5:3.5:0.2) for other fractions gave better resolution and maximum number of spots. Hence they were selected. The HPTLC profiles of different fractions are presented in Figure 4, 5, 7. PE showed 2 spots in both 254 nm and 366 nm at R_f 0.26 (22.57 %) and 0.28 (34.80 %). 2 spots were also observed after spraying with anisaldehyde sulphuric acid reagent. As compared to PE, ME revealed better separation and more number of spots in all the detecting systems. The densitogram of ME (Figure 6) showed 5 prominent spots at R_f 0.08 (4.56 %), 0.19 (65.37 %), 0.31 (7.85 %), 0.49 (3.90 %) and 0.67 (12.39 %). After spraying with different spray reagents like ferric chloride it showed two spots while with anisaldehyde sulphuric acid it showed 5-6 prominent spots. Observations indicate the presence of phenolic and flavonoid type compounds.

The chromatographic profiles of different fractions of ME have been presented in Figure 7. Maximum number of spots could be seen in chloroform fraction indicating more number of compounds is extracted in this fraction. Densitograms of CH (Figure 8) showed number of spots at different R_f values 0.17 (18.23 %), 0.28 (9.75 %), 0.43 (8.63 %), 0.62 (37.76 %) and 0.80 (23.15 %); similarly in EA, compounds separated on R_f 0.17 (37.39 %), 0.28 (8.20 %), 0.63 (21.40 %) and 0.69 (25.67 %) (Figure9). MES revealed 5 prominent spots at R_f value 0.07 (5.08 %), 0.17 (62.25 %), 0.28 (7.49 %), 0.44 (4.89 %) and 0.63 (12.34 %) (Figure10). This information will be very useful for isolation of active compounds from Panchagavya Ghrit. The 3D graph at 254 nm and 366 nm has beenpresented in Figure 11 and 12 respectively.

The inclination towards natural products based therapy is increasing throughout the world. Instances of adulteration have raised concerns regarding their wide use and are affecting their marketing. It is essential to standardise the products to ensure their quality. Since the natural products mostly contain number of chemical substances of different nature and their natural variation is also expected, it is very difficult to provide a comprehensive analytical protocol for their quality control. Hence, numbers of quality control parameters are used. In the present study Panchagavya Ghrit has been analysed by using various parameters. Different chromatographic profiles have also been evolved which may take care about substances of varied chemical groups present in it. The evolved data will be very useful for quality control of Panchagavya Ghrit.

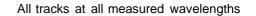
CONCLUSION:

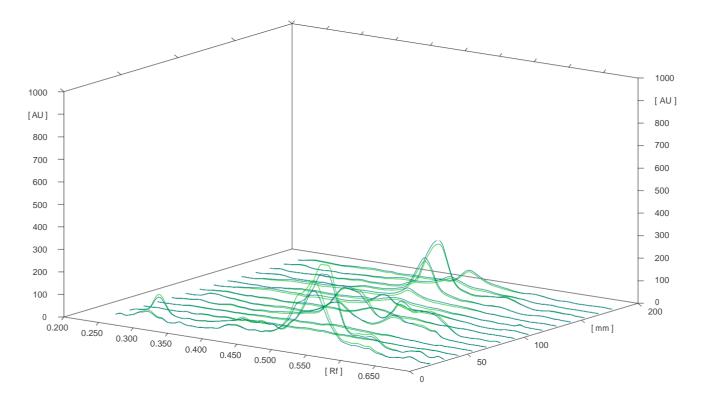
In the present communication the analytical profile of Panchagavya Ghrit, an Ayurvedic formulation has been presented. The analysis was carried out by using different physico-chemical, spectrophotometric and chromatographic methods. The evolved spectral data as well as the HPTLC profile will be very much useful for its quality control.

Test Parameter/ Sample	Results
Color	White
Odor	Characteristic
Taste	Characteristic
рН	4.80
Loss on drying @ 110° C	Not more than 0.4% W/W
Refractive index @ 40° C	1.349 to 1.345
Acid value	Not more than 3
Saponification value	201 to 226
Iodine value	34 to 43

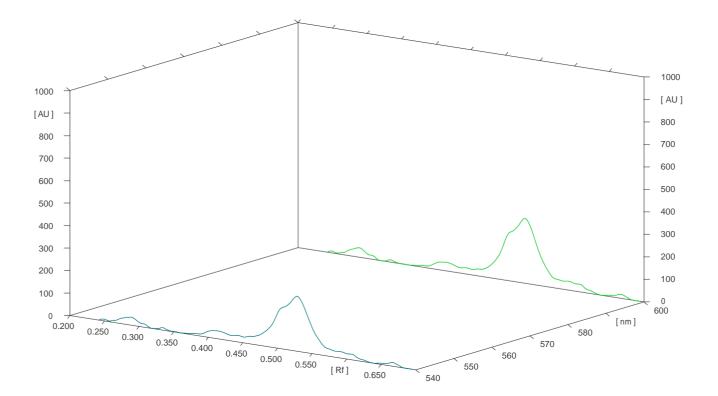
Table 1: Analytical data of Panchagavya Ghrit

Figures:



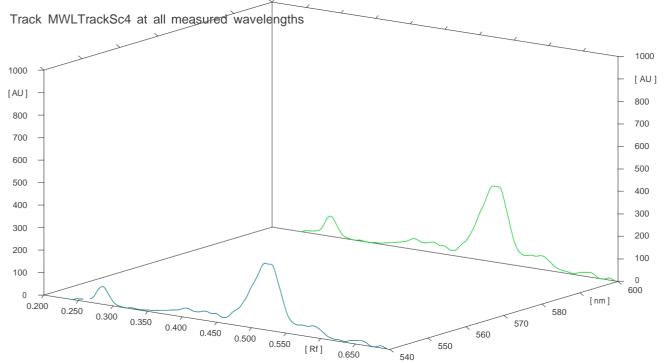


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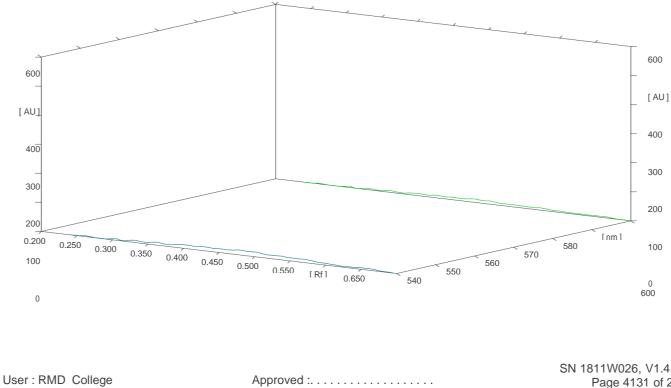


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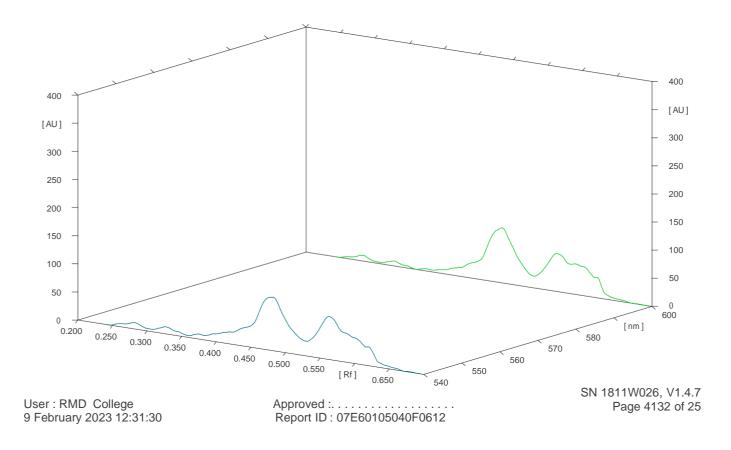
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700 700 [AU] [AU] 500 500 400 400 300 300 200 200 100 100 0 600 0 [nm] 0.200 0.250 0.300 0.350 580 570 0.400 0.450 560 0.500 0.550 550 [Rf] 0.650 540

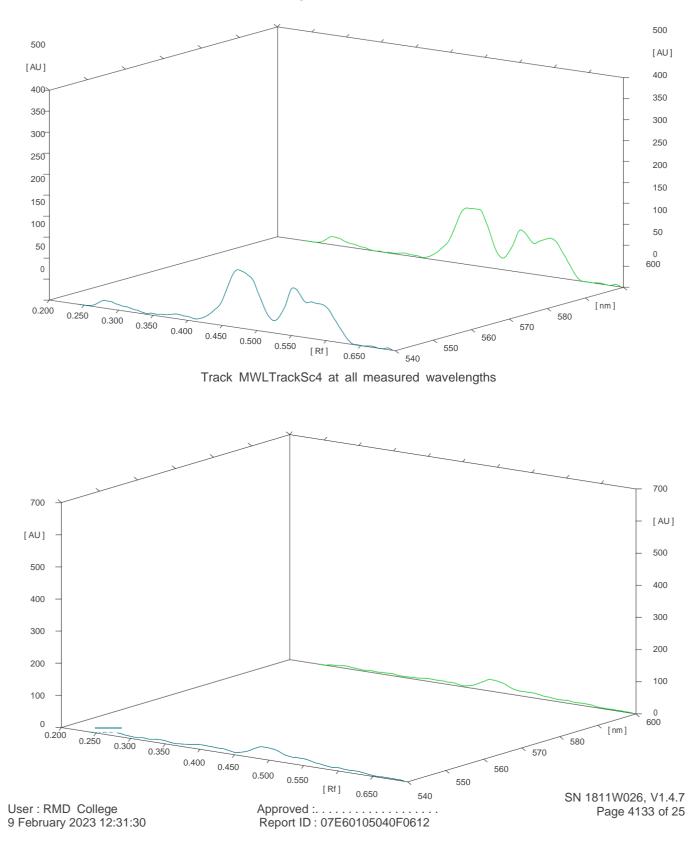
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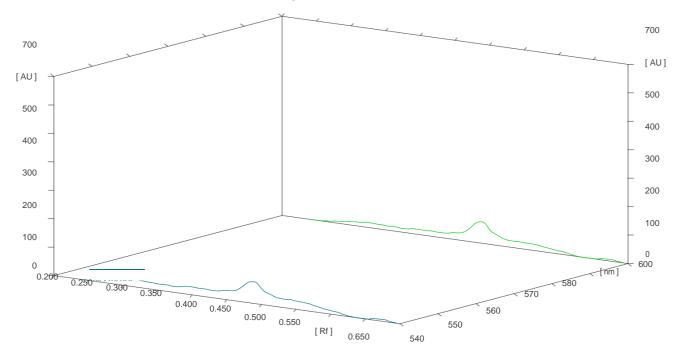


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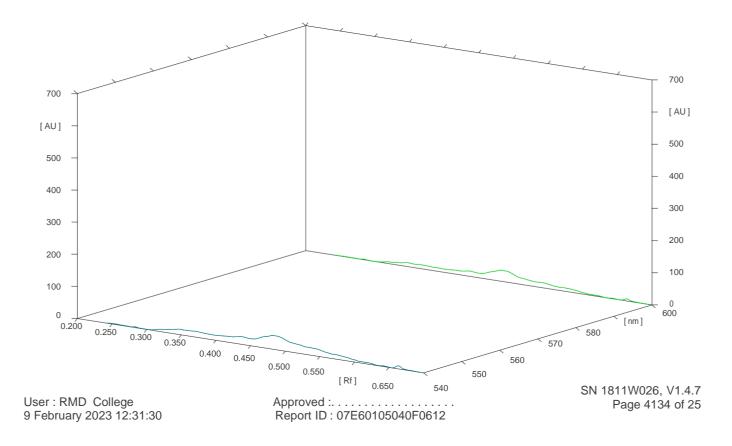
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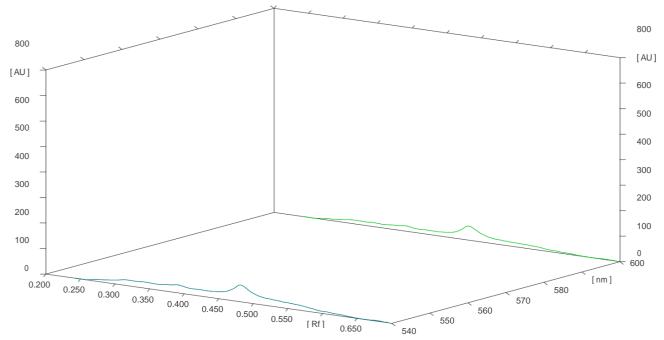
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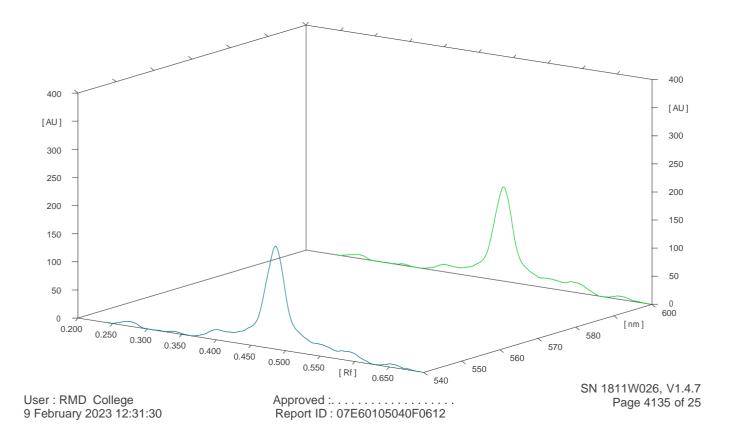


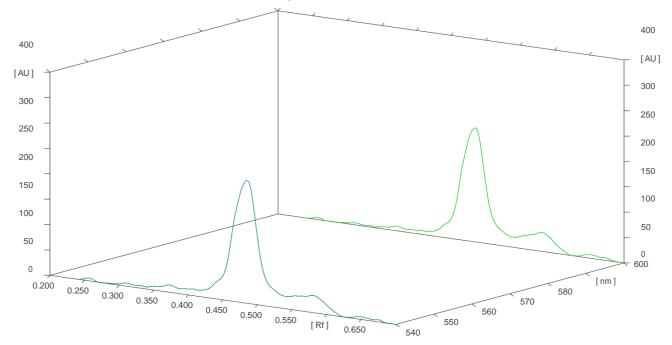
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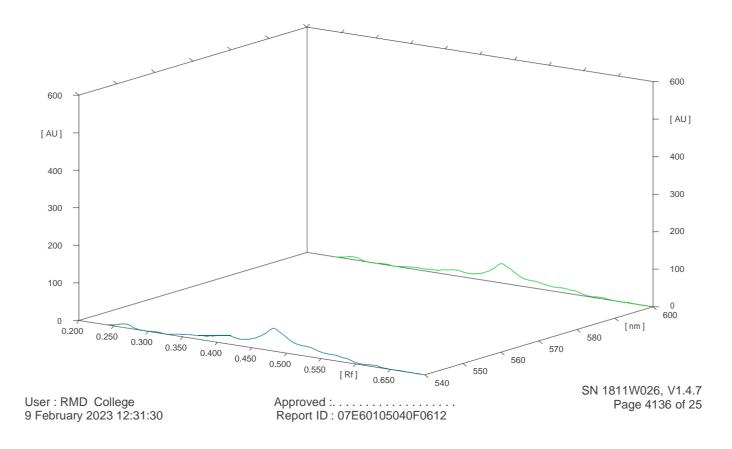






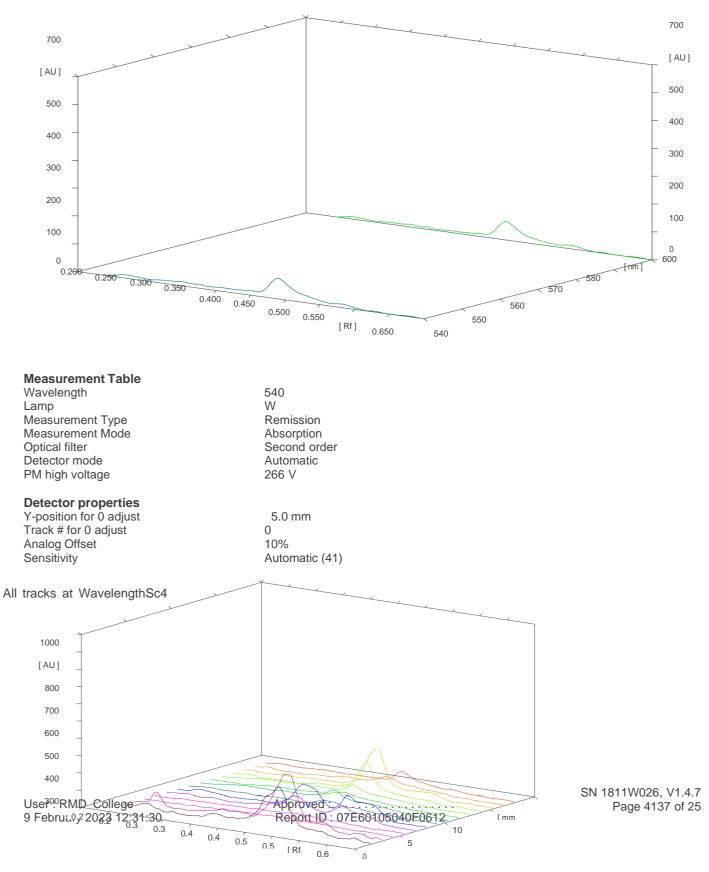
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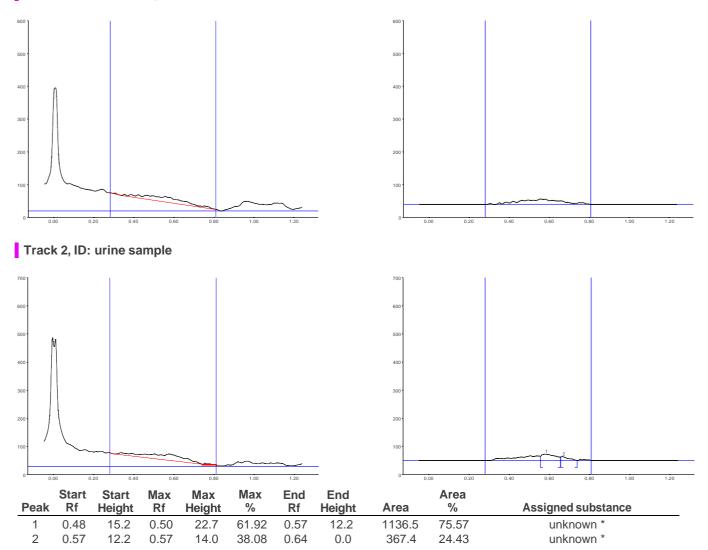
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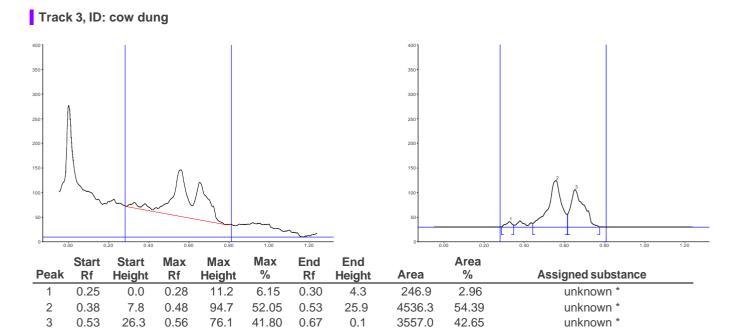


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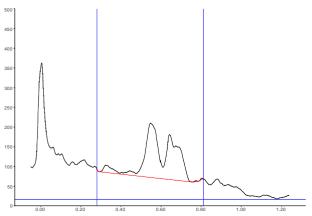


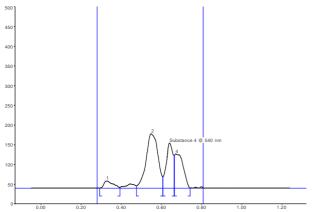


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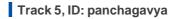


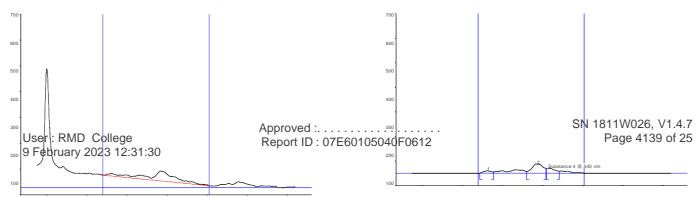
Track 4, ID: cow dung





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2	0.41	5.7	0.47	137.8	38.57	0.52	29.2	5878.8	49.07	unknown *
3	0.52	29.3	0.55	114.2	31.95	0.57	83.4	2788.1	23.27	Substance 4
4	0.57	83.8	0.58	87.0	24.35	0.64	0.3	2706.4	22.59	unknown *





0 0.00 0.20 0.40 0.60 0.80 1.00 1.20

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0.00

0.20

0.40

0.60

0.80

1.00

1.20

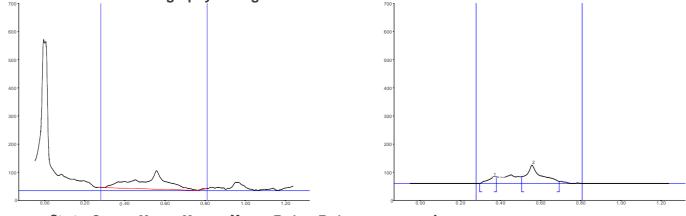
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Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	A	ssigned	substance	9	600
1	0.25	0.0	0.33	24.4	18.57	0.35	22.3	1030.6	18.71		unkn	iown *		—
2 3	0.43	25.2	0.49	76.0	5 <mark>7</mark> .80	0.53	29.9	3178.1	57.70		unkn	iown *		400
3	0.53	29.7	0.54	31.1	2 <mark>3</mark> .63	0.63	1.0	1299.2	23.59		unkn	iown *		300
200 100 0	0.00	0.20	0.40	0.60	0.80 1.		.20	200 100 0	0.00	0.20 0	0.40 0.6	0 0.80	1.00	1.20

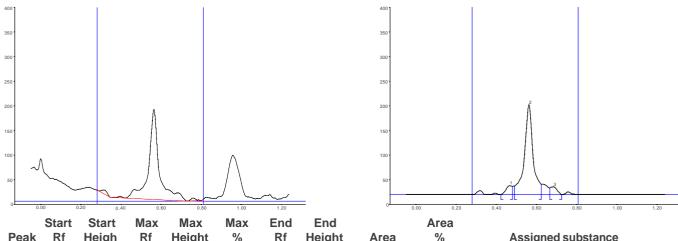
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Track 8, ID: milk winCATS Planar Chromatography Manager

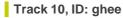


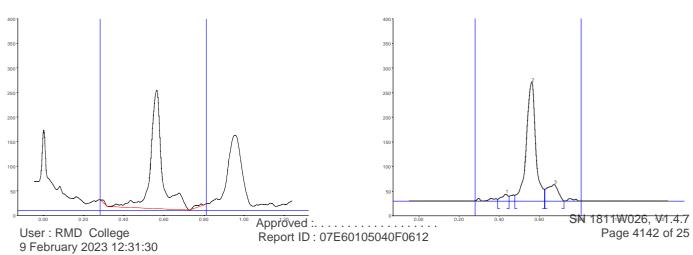
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Peak	Rf	Heigh	Rf	Height	%	Rf	Height	Area	%	Assigned substance
1	0.26	0.0	0.32	22.1	25.28	0.33	21.9	680.3	16.44	unknown *
2	0.44	24.4	0.48	65.2	74.72	0.60	8.0	3456.5	83.56	unknown *





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Peak	Rf	Heigh	Rf	Height	%	Rf	Height	Area	%	Assigned substance
1	0.37	0.4	0.40	18.3	8.43	0.42	16.9	403.1	6.21	unknown *
2	0.42	17.2	0.49	182.6	84.25	0.54	21.1	5702.5	87.80	unknown *
3	0.57	13.3	0.59	15.9	7.32	0.63	0.1	389.6	6.00	unknown *





n <mark>CAT</mark>	Start S Plan 0.34	Start Height	Max Rf	Max Height	Max Manage	End Rf	End Height	Area	Area %	Assigned substance
1		3.7	0.37	14.1	Hañage 4.88		9.8	342.6	3.47	unknown *
2 3	0.41	11.8	0.48	241.5	83.45	0.54	24.1	8183.9	83.00	unknown *
3	0.54	24.2	0.58	33.8	11.67	0.62	0.1	1333.6	13.53	unknown *
Track	(11, IC): curd								
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0.0	Start	Start	Max	Max	Max	End	End	0.	Area	0.40 0.00 0.00 1.00 1.2
Peak	Rf	Height	Rf	Height	%	Rf	Height	Area	%	Assigned substance
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2	0.43	17.9	0.48	60.4	72.52	0.52	26.2	2270.0	75.81	unknown *
): curd	0.10	00.4	12.52	0.02	20.2	2379.9	75.01	
				00.4			20.2	700 600 500 400 200 100	10.01	
Track	(12 , IE				1.00		-	700 600 500 400 200 100	00 0.20	
000	start	D: curd	o.éo Max		1.00 Max	1.20 End	End		00 0.20 Area	
Track	start Rf	D: curd		 Max Height	1.00 Max %	1.20 End Rf	End Height	700 600 500 400 200 100 0 0 Area	میں Area %	2 0.40 0.80 0.80 1.00 1.20
Track	start	D: curd	o.éo Max		1.00 Max	1.20 End	End		00 0.20 Area	

Wavelength
Lamp
Measurement Type
Measurement Mode
Optical filter
Detector mode
PM high voltage

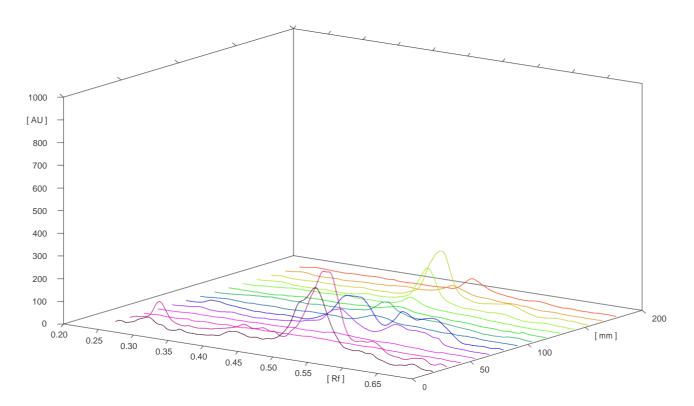
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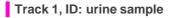
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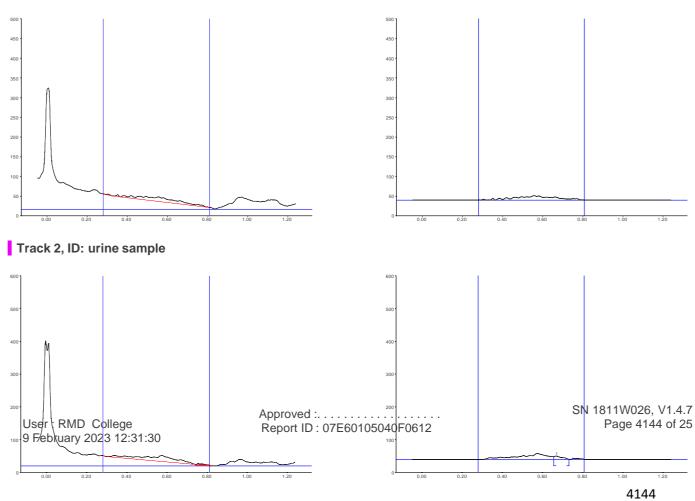
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All tracks at WavelengthSc4 winCATS Planar Chromatography Manager





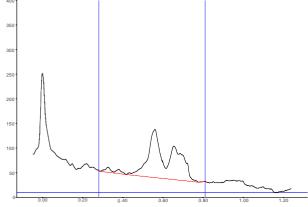


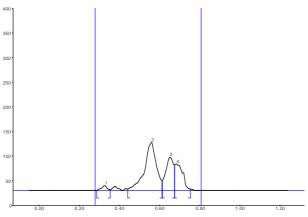
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				Height		Rf	Height	Area	%	Assigned substance
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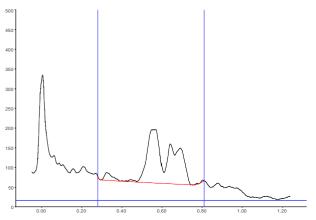
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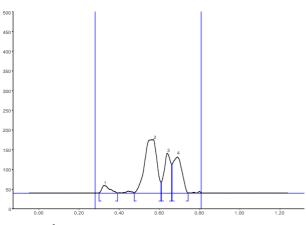




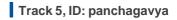
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2	0.38	3.1	0.48	97.9	42.55	0.53	21.0	4215.5	53.46	unknown *
3	0.53	21.2	0.56	67.5	29.37	0.58	52.3	1872.0	23.74	unknown *
4	0.58	52.5	0.59	54.3	23.61	0.65	3.3	1582.6	20.07	unknown *

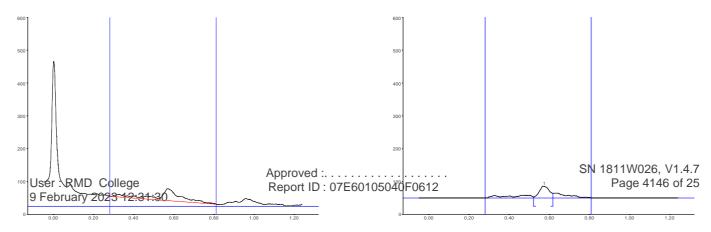
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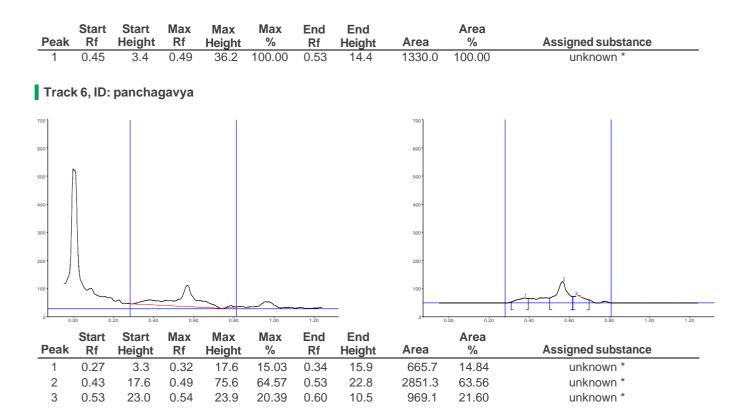




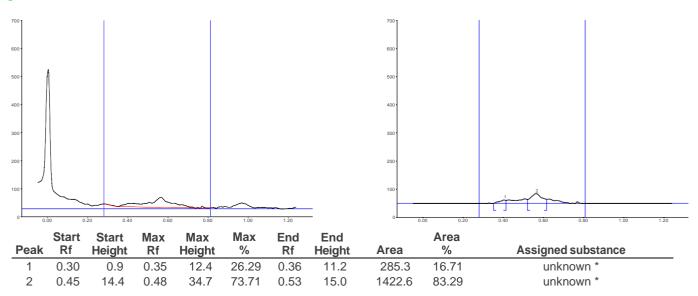
								-		
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.26	1.0	0.28	19.6	5.62	0.34	0.2	555.6	4.64	unknown *
2	0.41	1.2	0.49	135.8	39.02	0.52	27.7	6050.1	50.58	unknown *
3	0.53	28.1	0.55	101.1	29.04	0.57	73.5	2390.6	19.99	unknown *
4	0.57	73.7	0.59	91.7	26.33	0.64	0.4	2965.6	24.79	unknown *



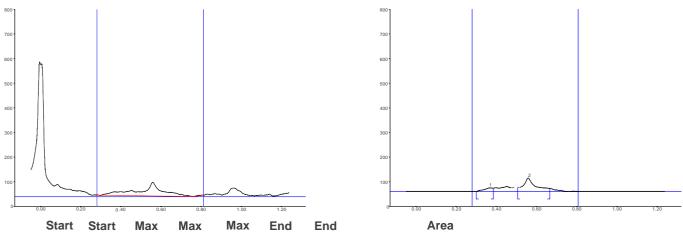




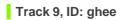
Track 7, ID: milk

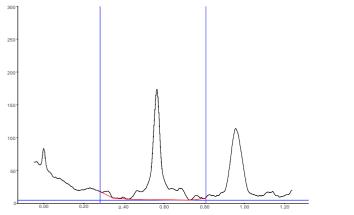


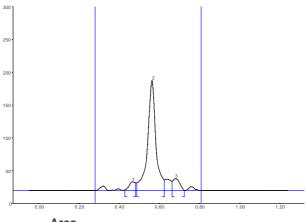
Track 8, ID: milk



Peak	Rf	Start Heigh	Max Rf	Max Height	wax %	End Rf	End Height	Area	Area %	Assigned substance
1	0.26	0.1	0.32	15.2	21.53	0.33	13.9	505.1	16.84	unknown *
2	0.44	16.1	0.48	55.4	78.47	0.57	12.8	2493.9	83.16	unknown *

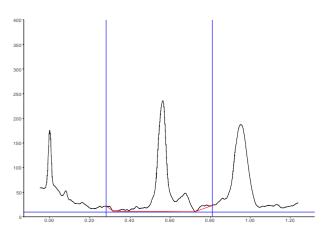


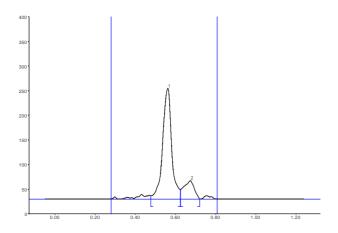




Peak	Start Rf	Start Heigh	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.37	0.9	0.40	13.2	6.64	0.42	11.7	282.4	4.95	unknown *
2	0.42	11.7	0.49	167.9	84.33	0.54	16.2	4972.5	87.13	unknown *
3	0.57	14.2	0.58	18.0	9.03	0.63	0.0	452.1	7.92	unknown *

Track 10, ID: ghee

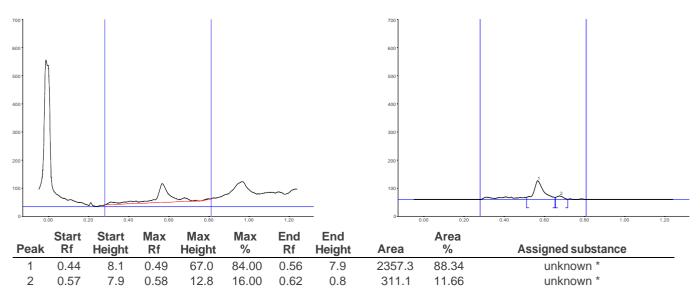




Eur. Chem. Bull. 2023, 12(Special Issue 8),4125-4153

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.41	6.7	0.49	224.8	85.72	0.54	19.9	7318.8	83.93	unknown *
2	0.54	19.9	0.58	37.4	14.28	0.62	0.2	1400.9	16.07	unknown *
Tracl	k 11, ID): curd								
-	,									
600								600		
500								500		
400								400		
300								300		
200								200		
100			^		~			100		$\sum_{i=1}^{2}$
		~~~	$\sim$	<u> </u>	$\sim$	$\sim$	_			
0.0	00 0.	.20 0.40	0.60	0.80	1.00	1.20	_	0.0.	00 0.20	0.40 0.60 0.80 1.00 1.20
	Start	Start	Мах	Max	Max	End	End		Area	
Peak	Rf	Height	Rf	Height	%	Rf	Height	Area	%	Assigned substance
1	0.39	7.0	0.42	15.6	19.61	0.43	11.9	417.1	15.88	unknown *
2	0.43	12.0	0.48	51.5	64.77	0.52	19.0	1877.6	71.51	unknown *
3	0.56	11.2	0.57	12.4	15.61	0.61	1.7	331.1	12.61	unknown *

Track 12, ID: curd



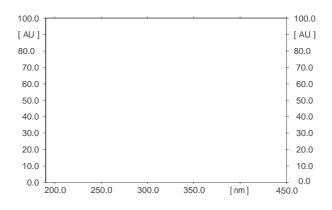
#### Spectrum scan

Executed by
Mode
Slit dimensions
Optimize optical system
Scanning speed
Data resolution
Reference spectrum, pos X
Reference spectrum, pos Y

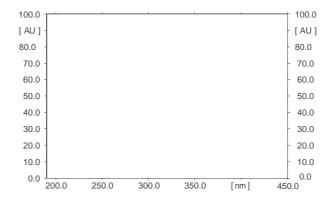
RMD College05 January 2022 15:02:30All detected peaks6.00 x 0.45 mm, MicroLight100 nm/s1 nm/step10.0 mm10.0 mm

Measurement Table Lamp Start wavelength End wavelength Measurement type Measurement Mode Optical filter Detector Mode	D2 190 nm 450 nm Remission Absorption Second order Automatic
Detector properties Y-position for 0 adjust Track # for 0 adjust Analog Offset Sensitivity	0.0 mm 0 10% Automatic (0)

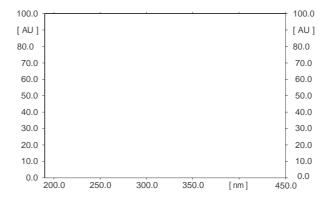
#### Substance 1 on all Tracks



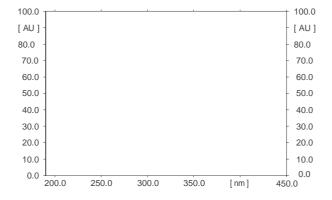
#### Substance 2 on all Tracks



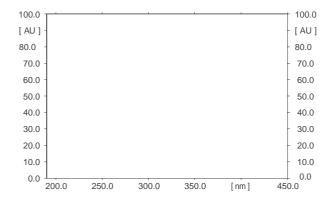
#### Substance 3 on all Tracks



#### Substance 4 on all Tracks



#### Substance 5 on all Tracks



#### **Evaluation results**

# **Evaluation Sequence**

Track	Track type	Vial	Sample ID
1	Sample	1	urine sample
2	Sample	1	urine sample
3	Sample	2	cow dung
4	Sample	2	cow dung
5	Sample	3	panchagavya
6	Sample	3	panchagavya
7	Sample	4	milk
8	Sample	4	milk
9	Sample	5	ghee
10	Sample	5	ghee
11	Sample	6	curd
12	Sample	6	curd

#### Table of substances

	Position Tracks	1			
Substance	MDmm 1 2 3 4 5	678901234			
Substance 1	67.4				
Substance 2	63.7				
Substance 3	57.5				
Substance 4	47.6				
Substance 5	40.5				

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