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Simultaneous Determination of Nicotine, 1,2-Propylene Glycol and Glycerol in E-liquids by Gas Chromatography Method

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Introduction

- **Nicotine is an essential ingredient in e-liquids.**
  - **Declarations of nicotine levels contained in e-liquids have been found to be incorrect. Varying amounts of nicotine were detected in e-liquids declared as nicotine-free.**
  - **Intake a high level of nicotine may result in serious symptoms of nicotine poisoning.**
  - **Directive 2014/40/EU: nicotine concentration does not exceed 20 mg/ml.**

- **Propylene glycol (PG) and glycerol are used in e-cigarettes to produce visible vapor, which is the vehicle for nicotine and flavors. E-liquids usually contain about 90% of either propylene glycol, glycerol, or a mix of both substances.**

- **There is no international standardized method for the determination of nicotine, propylene glycol and glycerol content in e-liquids.**

Objectives

- Development of a sufficiently robust testing method will meet regulators needs.
- Assist producers and distributors in assessing their products.
- And protect consumers from substandard products.

Literature Methods

- **Nicotine**
  - ✓ **Sample Preparation: Solvent dilution or extraction**
  - ✓ **Determination: GC-FID/MS, HPLC, LC-MS, etc.**
- **Propylene Glycol & Glycerol**
  - ✓ **Sample Preparation: Solvent dilution**
  - ✓ **Determination: GC-FID/MS, HPLC, etc.**

ZTRI Method

Sample preparation

Weigh 0.1g of e-liquid sample into a 50 mL conical flask

Add 10 ml of isopropanol (0.2 mg/ml of quinaldine and 1 mg/ml of 1,3-butanediol) into the flask

Shake for 20 min

GC-FID analysis

ZTRI Method

GC Conditions

Instrument	**Agilent 6890 GC, equipped with a flame ionization detector**
Column	**DB-ALC1（ 30m×0.32mm×1.8μm）**
Oven temperature	**100℃ (1 min), 15 ℃/min to 130℃, 40℃/min to 220 ℃(10 min)**
Injection temperature	**250 ℃**
Injection volume	**1 μL**
Injection mode	**Split, 50:1**
Carrier gas	**He, at a flow rate of 1.8 ml/min**
Detector temperature	**275 ℃**
Air	**450 ml/min**
H_2	**40 ml/min**

ZTRI Method

Chromatograms of standard and sample

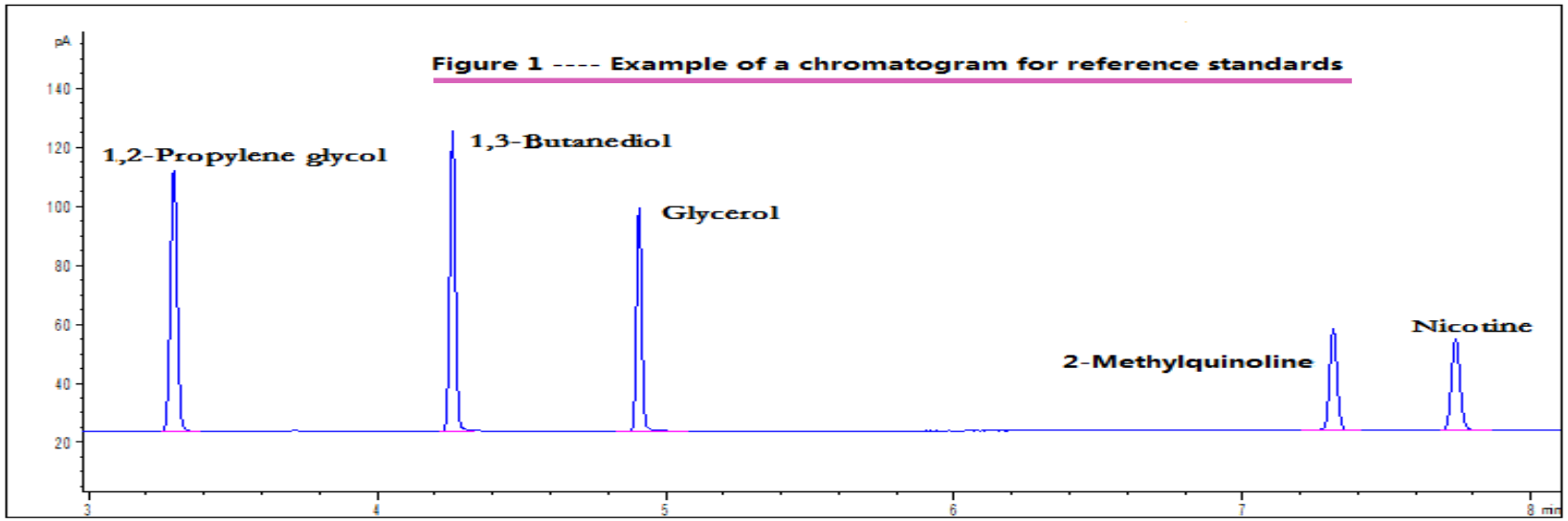


Figure 1 ---- Example of a chromatogram for reference standards

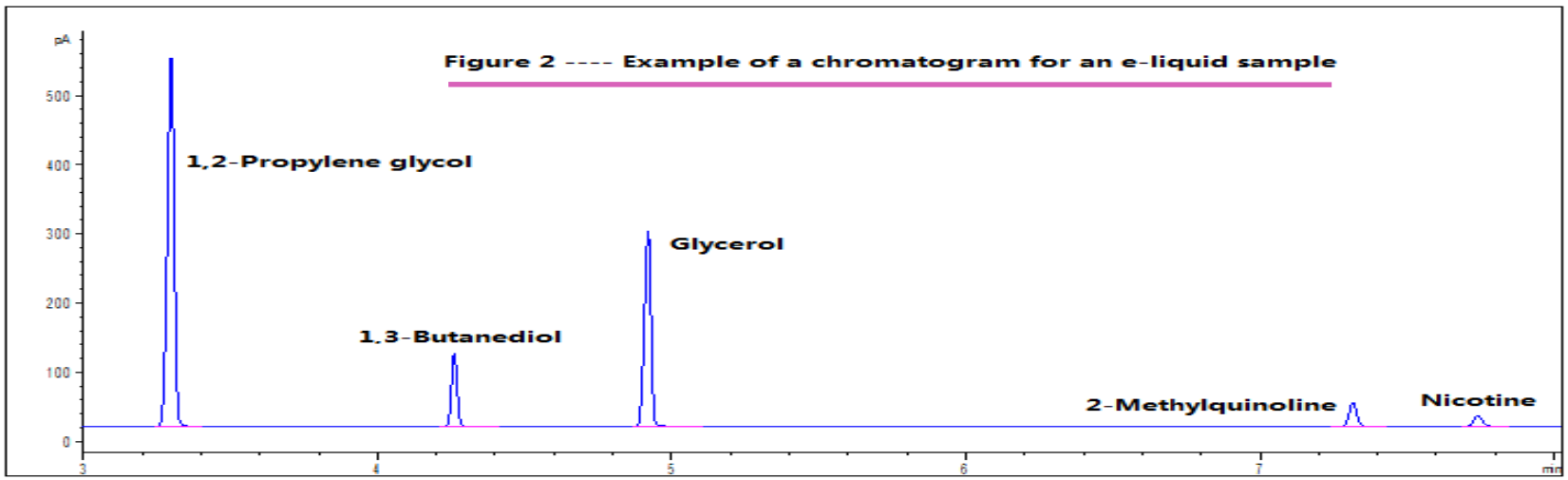


Figure 2 ---- Example of a chromatogram for an e-liquid sample

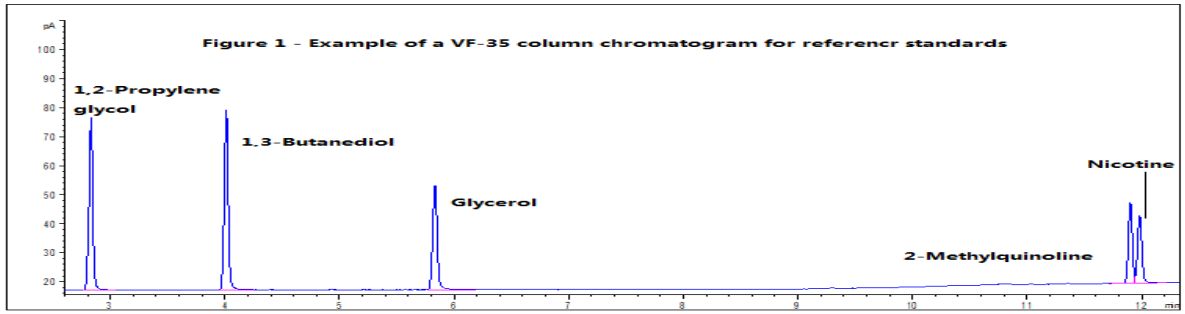
Results and Discussion

Optimization of the Sample Preparation

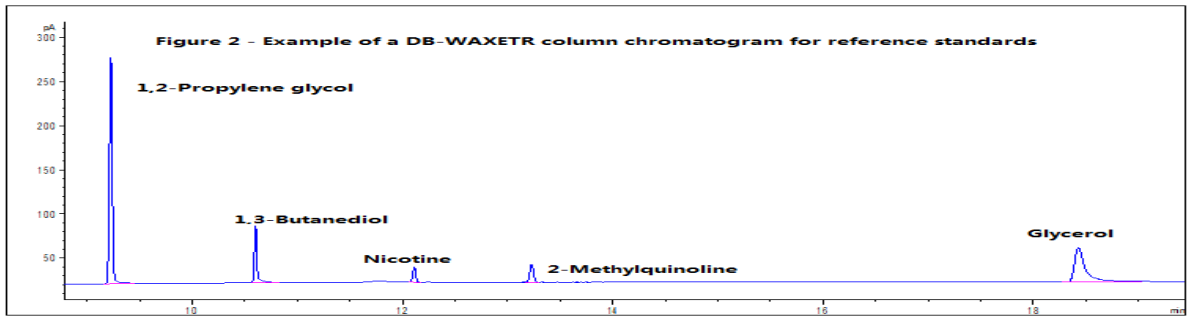
- **Internal Standards:**
  - ✓ **Nicotine:**
    - **Quinaldine** (CORESTA Recommended Method N° 62 and N° 66)
    - **Heptadecane** (CORESTA Recommended Method N° 9, N° 62 and N° 66) (Alternative IS)
  - ✓ **Propylene glycol and glycerol:**
    - **1,3-Butanediol** (CORESTA Recommended Method N° 60)
    - **1,4-Butanediol** (CORESTA Recommended Method N° 60) (Alternative IS)

Results and Discussion

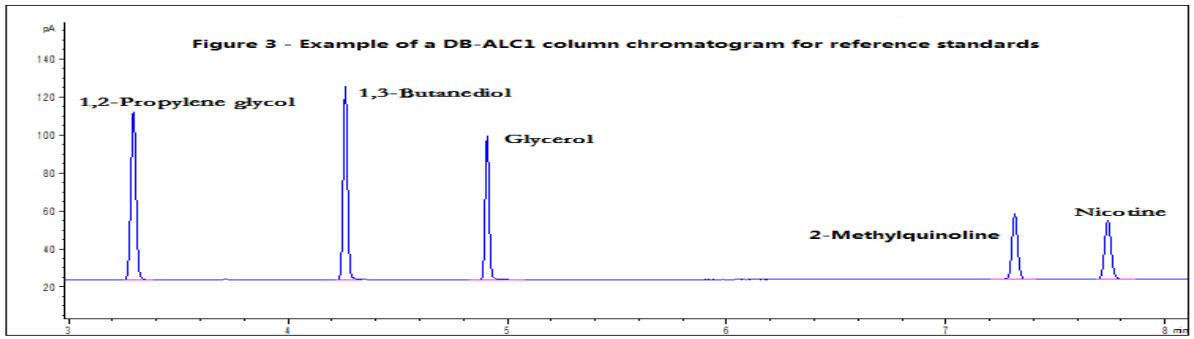
Column Selection



VF-35
30m×0.32mm×1.0μm



DB-WAXETRE
30m×0.32mm×1.0μm



DB-ALC1
30m×0.32mm×1.8μm

Results and Discussion

Optimization of the Sample Preparation

- **Dilution Solvents:**

  Isopropanol, Methanol

- **Shaking Time:**

Dilution Solvent	Shaking Time, min	Nicotine, mg/g	PG, mg/g	Glycerol, mg/g
Isopropanol	5	6.11	581	398
	10	6.12	581	398
	15	6.13	583	401
	20	6.15	583	402
	25	6.15	584	401
	30	6.14	585	400
Average		6.13	583	400
CV, %		0.9	0.3	0.5

Results and Discussion

Linearity

Compound	Linear range mg/ml	Equation	R^2	LOD μg/ml	LOQ μg/ml
Nicotine	0.01 – 0.4	y = 0.8274x + 0.0009	0.9999	1.3	4.3
PG	0.10 - 10	y = 0.8516x + 0.0031	0.9999	0.9	3.0
Glycerol	0.10 - 10	y = 0.6706x - 0.0135	0.9998	3.1	10.4

Results and Discussion

Precision

Precision		Nicotine	PG	Glycerol
Intra day	Mean, mg/g	6.09	576	405
	RSD, %	0.6	0.4	0.4
Inter day	Mean, mg/g	6.10	578	400
	RSD, %	1.6	0.6	1.1

Results and Discussion

Recovery

- **Nicotine：97.4%～101.6%**
- **Propylene Glycol：96.4%～100.0%**
- **Glycerol：100.0%～102.4%**

Results and Discussion

Collaborative Study

- **Samples**

No.	Flavor	Labelled Nicotine Level
Sample 1	American Tobacco	11 mg
Sample 2	Green Tea	8 mg
Sample 3	Chinese Virginia	High
Sample 4	Cigar	High
Sample 5	Menthol	6 mg
Sample 6	Coke	0 mg

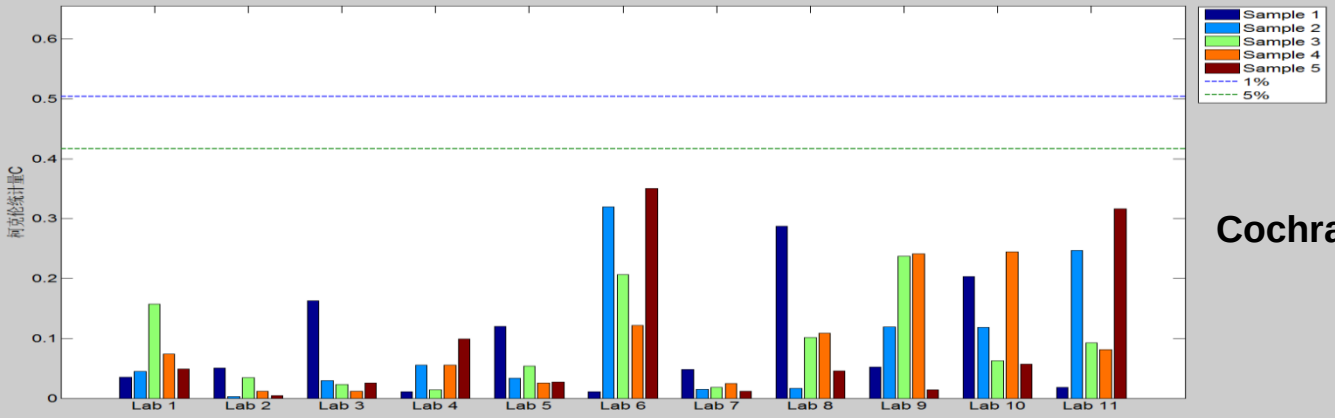
- **Study Plan**

  5 days, 2 determination per day

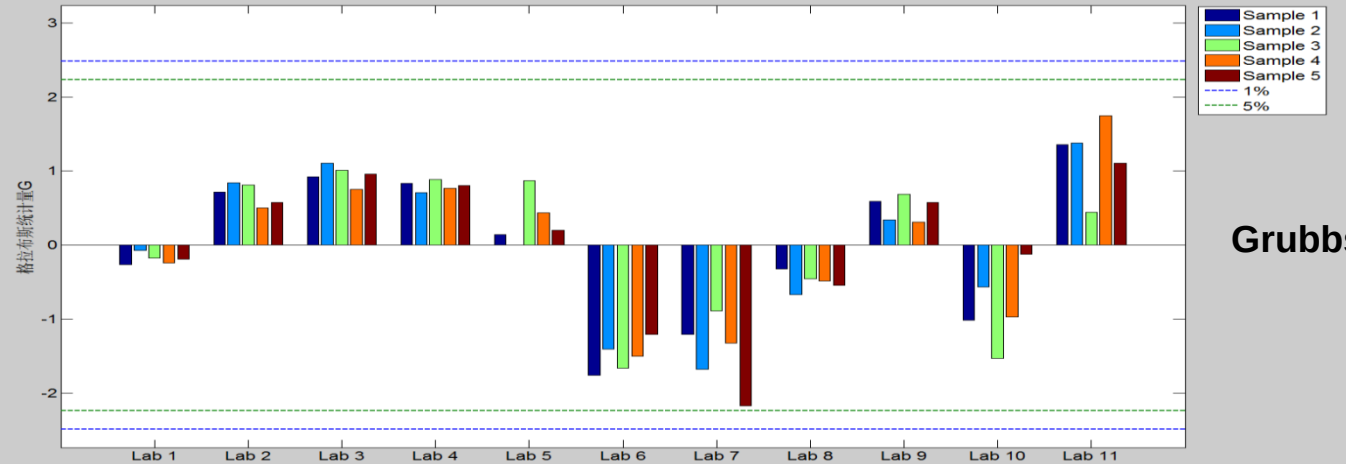
- **Participating Laboratories: 11**

Collaborative Study

Nicotine



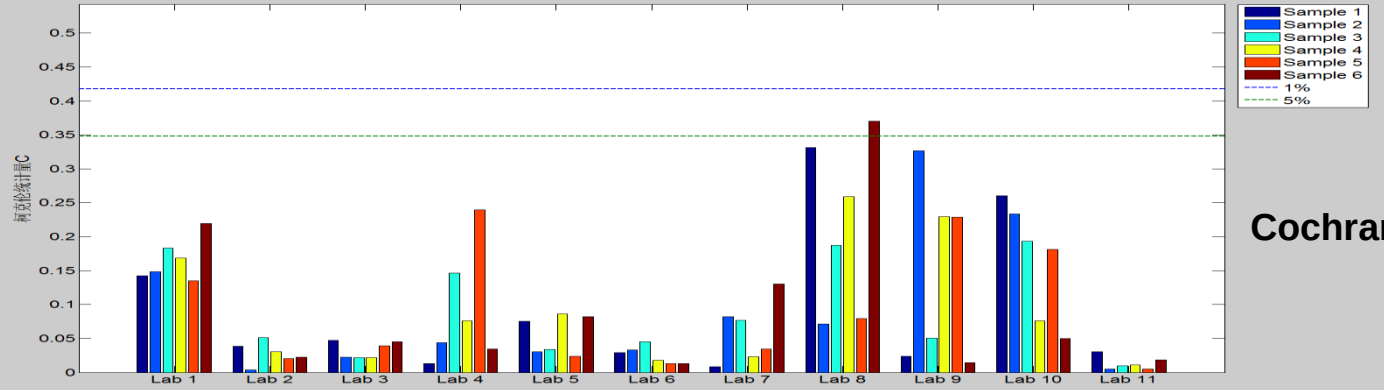
Cochran's Test



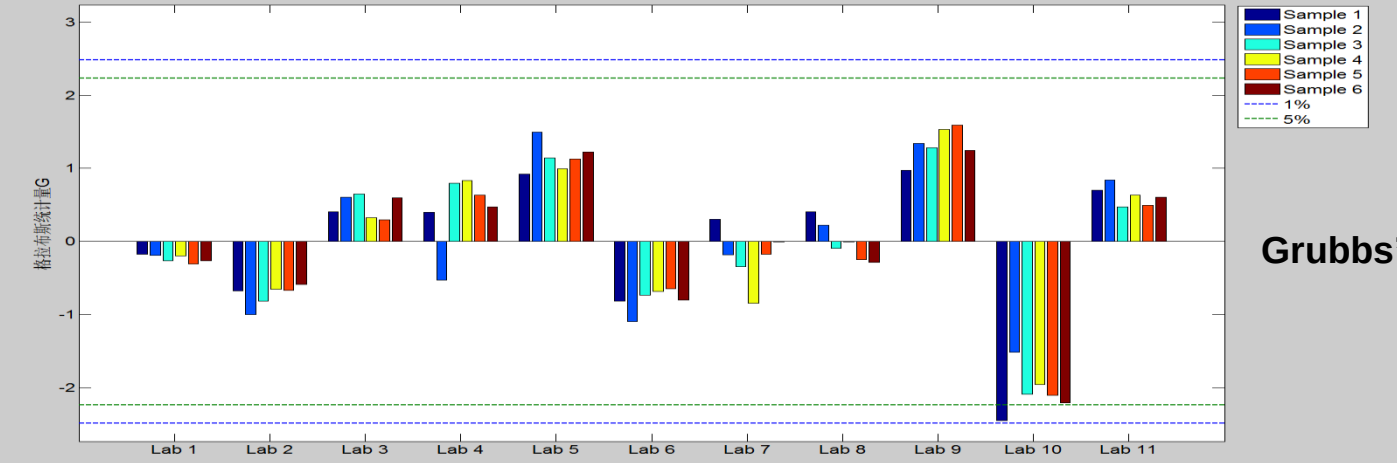
Grubbs' Test

Collaborative Study

Propylene Glycol



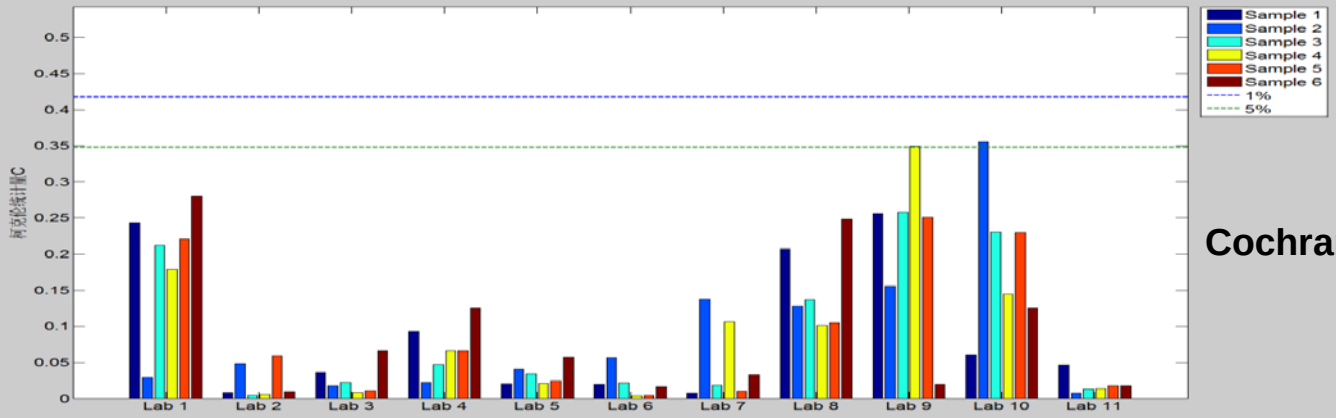
Cochran's Test



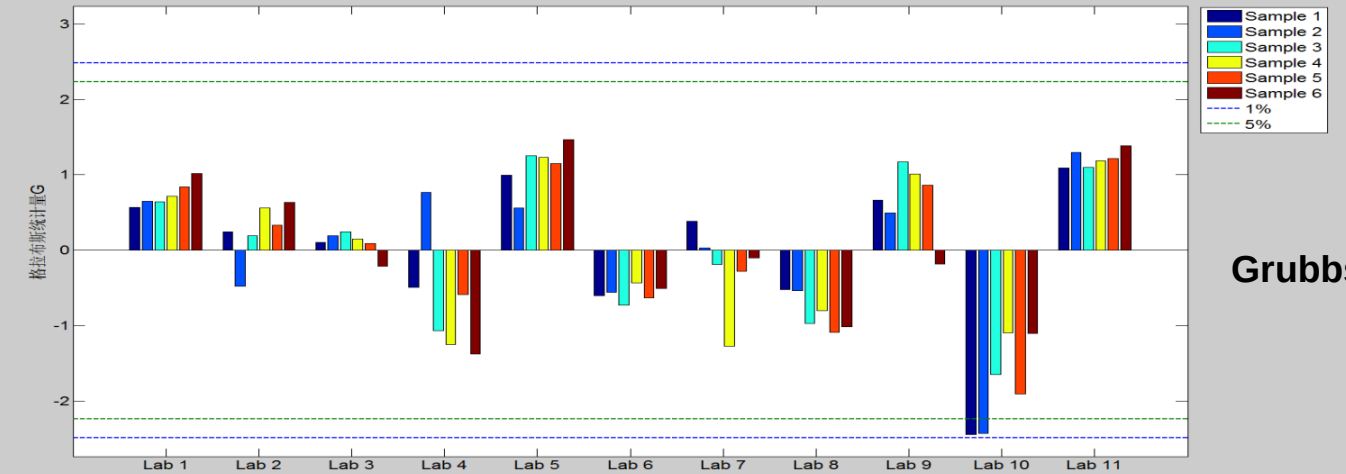
Grubbs' Test

Collaborative Study

Glycerol



Cochran's Test

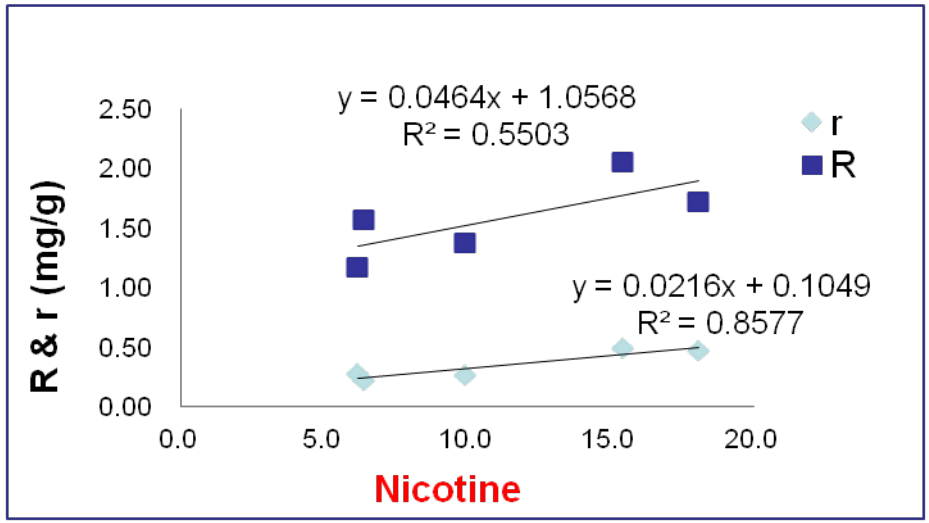


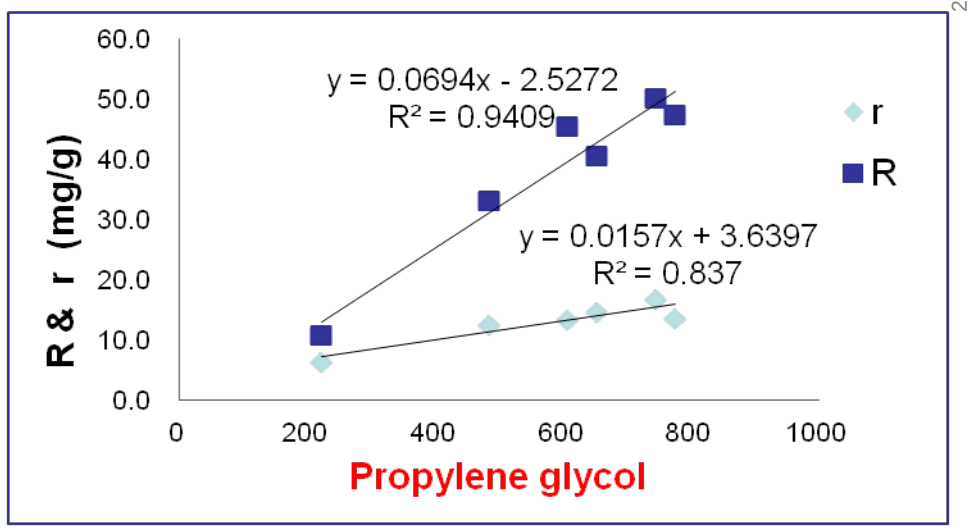
Grubbs' Test

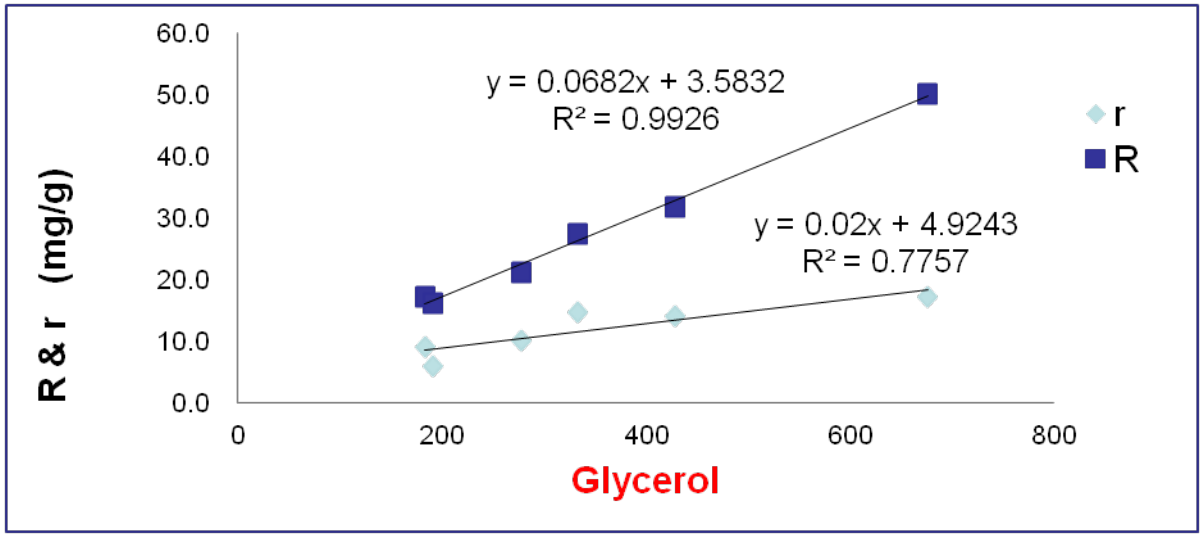
Repeatability & Reproducibility

		1#	2#	3#	4#	5#	6#
Nicotine	Mean, mg/g	9.93	6.17	18.1	15.4	6.39	0.00
	S_r/mean, %	0.9	1.6	0.9	1.1	1.3	-
	S_R/mean, %	4.9	6.7	3.4	4.7	8.7	-
	r, mg/g	0.27	0.28	0.47	0.50	0.23	0.00
	R, mg/g	1.38	1.17	1.72	2.05	1.57	0.00
PG	Mean, mg/g	608	223	654	746	485	776
	S_r/mean, %	0.8	1.0	0.8	0.8	0.9	0.6
	S_R/mean, %	2.6	1.7	2.2	2.4	2.4	2.2
	r, mg/g	13.2	6.3	14.5	16.7	12.4	13.4
	R, mg/g	45.3	10.6	40.6	50.0	33.1	47.3
Glycerol	Mean, mg/g	333	676	278	183	428	191
	S_r/mean, %	1.6	0.9	1.3	1.8	1.2	1.1
	S_R/mean, %	2.9	2.6	2.7	3.3	2.6	3.0
	r, mg/g	14.7	17.3	10.2	9.1	14.2	5.9
	R, mg/g	27.5	50.1	21.2	17.3	31.7	16.1

Repeatability & Reproducibility







Method Application

Flavor	Nicotine Labelled	Nicotine Measured, mg/g	Nicotine Deviation, %	PG, mg/g	Glycerol, mg/g
Cola	0 mg	0	-	783	192
Strawberry	0 mg	0	-	170	710
Tobacco	0 mg	0	-	0	918
Menthol	0 mg	13.6	-	745	191
Tobacco	6 mg	6.1	2.3	578	395
Green Tea	8 mg	6.9	-13.9	226	682
American Tobacco	11 mg	10.5	-4.6	613	337
Ice Blue	12 mg	6.6	-45	238	664
Classic	16 mg	9.9	-38.1	221	700
Cigar	High	15.9	-	749	185
Menthol	3.0%	23.7	-21.1	0	866
Rich Tobacco	4.5%	34.5	-23.3	0	856

Nicotine contents of some samples were inconsistent with the labeling data.

Conclusions

- **ZTRI method**
  - ✓ **Quick, easy, accurate and sensitive.**
  - ✓ **Good repeatability & reproducibility.**

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Thanks for your attention!