"TLC for pharmaceutical analysis in resource limited countries"

Eliangiringa Kaale,[1] Peter Risha[1] and Thomas Layloff[2]
1 Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania;
2 Supply Chain Management System, Arlington, VA.

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Overview of Chromatography 1/2

- Chromatography has become the mainstay of organic analysis because it greatly simplifies both **qualitative** and **quantitative assessment**s

- Technological advancement $\Rightarrow$ higher pressure liquid systems, more uniform and smaller particle stationary phases, modified stationary media, more robust and repeatable sample introduction systems

- Significant technology infrastructure to sustain them including spare parts inventories and skilled technicians to perform **repairs, corrective** and **preventive** maintenances and updates

Overview of Chromatography 2/2

- The more sophisticated systems become the more **significant sustainability issues arises**.

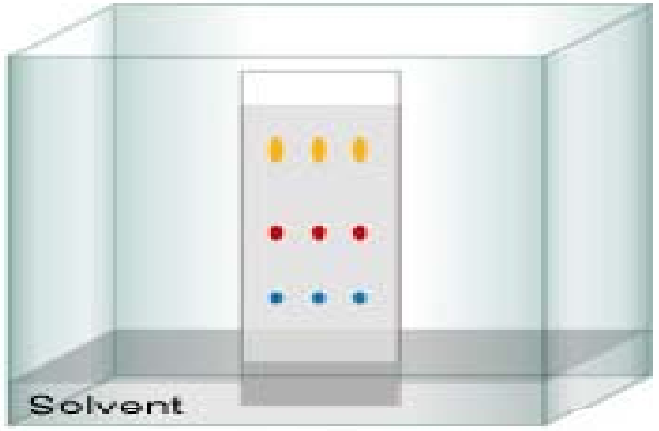
- Sustainability is a measure of the **probability of a system failing** and the **availability of resources** required restoring it to an operational state;

- The **lower the probability** of failure and/or the greater the **availability of restoration resources** the more sustainable.

Moving Things from Point A to Point B
Mechanical Technology Sustainability

Adult Tricycle

Rav 4



After Ten Minutes

Selection of Test Methods

- HPLC technology
  - HPLC is **currently the state** of art equipment for assay, dissolution and content uniformity testing in most pharmacopoeial monographs
- Demonstrate:
  - **Excellent robustness, accuracy, precision, selectivity, sensitivity** and also **linearity and range up 3 orders of** magnitude.
  - On the other hand –high consumption of **expensive**, ‘’**time consuming’’-** one sample at time,
- Resource constrained setting?

Overview of Chromatography 2/2

- TLC and the closely related (HPTLC) are members of a class planar chromatography where the separation solvent flow is **driven by capillary action**.
- Requires no **pumps, valves, pressure controls**, etc., so the chromatography systems are much more sustainable in any world market

HPTLC application in Africa **1/2**

Method Developed and validated Via SLV

- Shahista Hasan, Development and Validation of An HPTLC Densitometric Method for **Quantitative Analysis of Metronidazole** in Tablets, Unpublished student thesis work, MUHAS, (2007).
- Michael Sinda, Development and Validation of An HPTLC Densitometric Method for **assay of Quinine in Tablets**, Unpublished student thesis work, MUHAS, (2007).
- Jane Evarist, Development and Validation of An HPTLC Densitometric Method for **assay of Nevirapine in Tablets**, Unpublished student thesis work, MUHAS, (2007).
- Ikombola Juma, Development and Validation of An HPTLC Densitometric Method for simultaneous **assay of Lamivudine and zidovudine**
- Meshaki Shilinde, Development and Validation of An HPTLC Densitometric Method for **assay of Amodiaquine in Tablets**, Unpublished student thesis work, MUHAS, (2007).
- Mufaddal .S. Hassanali, Development and Validation of An HPTLC Densitometric Method for **Analysis of Ciprofloxacin** in Commercial Formulations, Unpublished student thesis work, MUHAS, (2007).

Improving the TLC based Testing methods

- What has been done
  - TFDA, SOP and MSH have collaboratively worked to develop and validate HPTLC- Densitometry **methods for assay** of selected essential medicines.
  - MUHAS-MSH-CAMAG – demonstrated **transferability of the methods- JAOAC –two lab Peer Method Verification**

JAOAC Peer Method Verification approach (PMV)

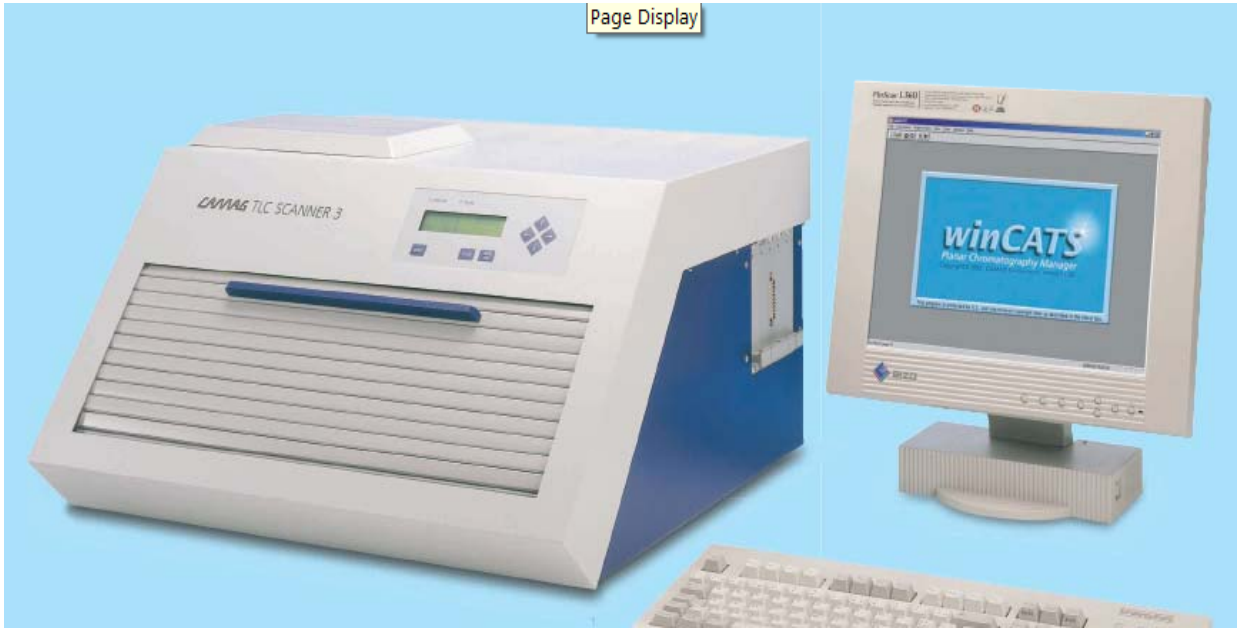
Setting up inter-laboratory cross validation

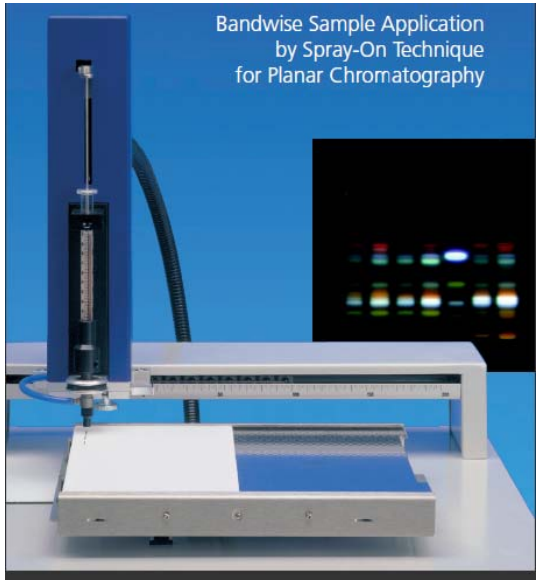
- MUHAS- originator
- CAMAG lab-
  - Receiving laboratory performed the method
  - To demonstrate where there are differences and gaps in documentation
    - ■Lack of detailed test method instructions
      - □Assay Conditions
      - □Calculations
      - □System Suitability
  - Differences with instrumentation

Method Transfer Protocol

- Test Method Example
  - Parameters being assessed
    - Precision, specificity, etc.
  - Sample Preparation/Reagent
  - Performance Parameters
    - Different analyst on different days
- Pre-defined Acceptance Criteria
- Statistical Analysis
- Sign-off by Quality Assurance Unit

HPTLC-densitometer





- **Metronidazole- qualifying**
- **Nevirapine**
- **Quinine**
- **Lamivudine/zidovudine**

2-lab collaborative

Summary conditions

Table 1 The developing solvent mixture ratios are presented in the table below					
Product	Developing Solvent Mixture Ratio				
	Ethyl Acetate	Toluene	Methanol	Ammonia (25%)	Acetone
Lamivudine-Zidovudine	12	5	3	--	--
Metronidazole	50	--	--	1	--
Nevirapine	15	5	--	--	--
Quinine	22	--	--	3	5

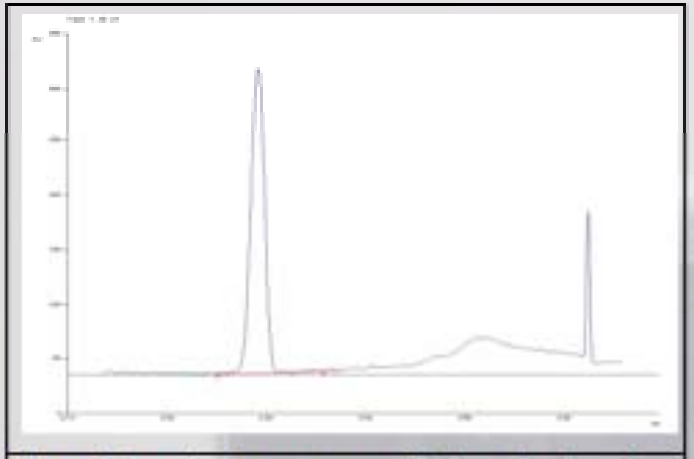


Figure1. A typical denstogram of 5 µL application of 0.075 µg/µL metronidazole solution. Mobile phase ethyl acetate and ammonia (25 : 0.5) v/v acquired by UV detection at 313 nm

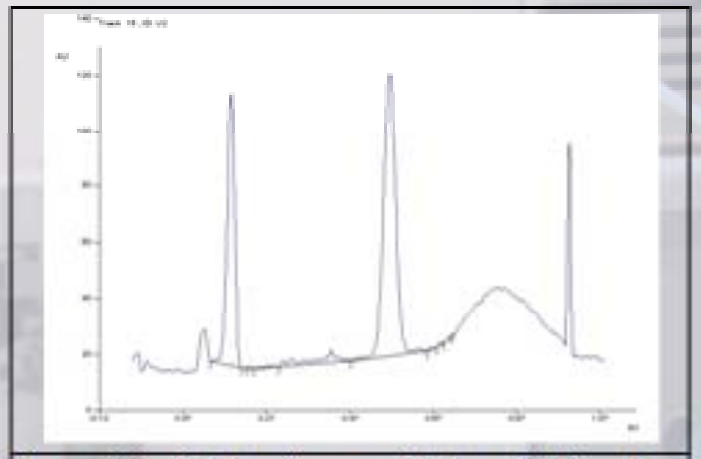


Figure 2. Typical densitogram of 5 µL application of 0.135 µg/µL Lamivudine/zidovudine solution. Mobile phase: ethyl acetate: toluene: methanol (12:5:3 v/v) acquired by UV detection at 289 nm

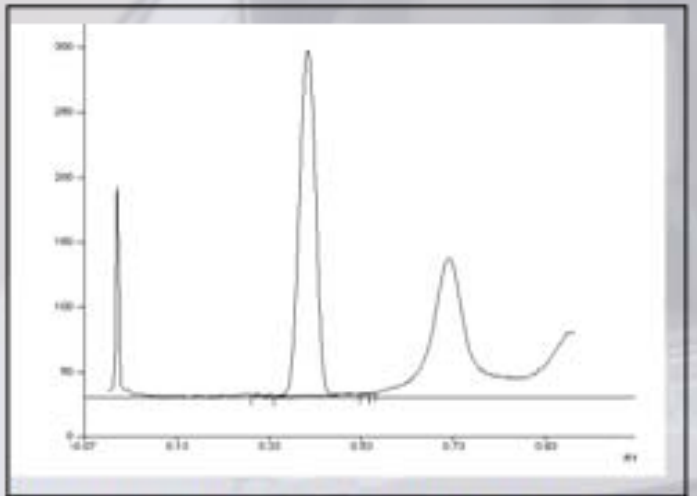


Figure 3 A typical densitogram of 5 µL application of 0.1 µg/µL Nevirapine mobile phase: ethyl acetate: toluene (7.5:2.5v/v) acquired by UV detection at 289 nm

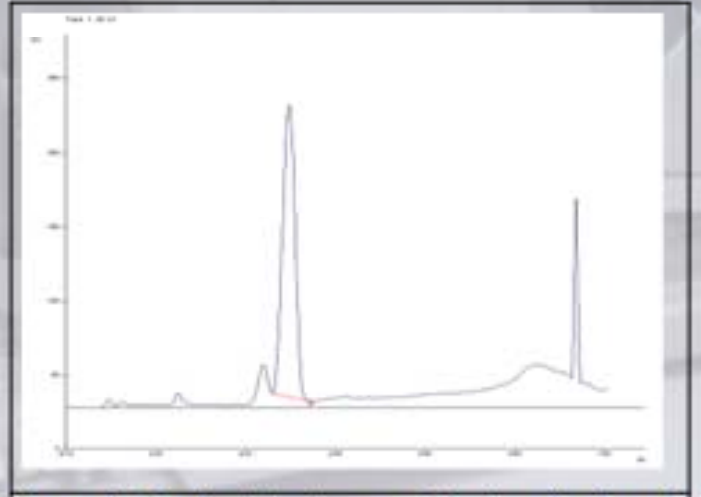


Figure. 4. A typical densitogram of 5 µL application of 0.25 µg/µL Quinine solution mobile phase ethyl acetate, acetone and ammonia (73%, 17%,3% v/v/v) acquired by UV detection at UV 327 nm

Table 2. Polynomial Correlations: 50-80-100-120-150%					
		MUHAS		CAMAG	
		Correlation Coefficient	rsd%	Correlation coefficient	rsd%
Lamivudine	Plate 1	0.99889	2.17	0.99919	1.95
	Plate 2	0.99991	0.64	0.99976	1.10
	Plate 3	0.99865	2.36	0.99989	0.72
Metronidazole	Plate 1	0.99967	0.90	0.99919	1.65
	Plate 2	0.99998	0.23	0.99918	1.66
	Plate 3	0.99948	1.18	0.99971	0.99
Nevirapine	Plate 1	0.99955	1.26	0.99990	0.63
	Plate 2	0.99940	1.50	0.99966	1.16
	Plate 3	0.99977	0.93	1.00000	0.14
Quinine	Plate 1	0.99924	1.50	0.99979	0.83
	Plate 2	0.99994	0.43	0.99991	0.53
	Plate 3	0.99972	1.26	0.99978	0.88
Zidovudine	Plate 1	0.99958	1.31	0.99945	1.84
	Plate 2	0.99953	1.40	0.99989	0.76
	Plate 3	0.99957	1.27	0.99960	1.43
Overall Averages		**0.99915**	**1.20**	**0.99966**	**1.02**

Table . 3 Metronidazole- Study qualifying				
MUHAS			CAMAG	
Average	99.41%		Average	97.81%
rsd	2.08%		rsd	1.81%
Interlaboratory Summary				
Average	98.61%		RSD	2.09%

Table 4. Quinine				
Summary				
MUHAS			CAMAG	
Average	95.67 %		Average	98.34 %
rsd	2.17%		rsd	1.78%
Interlaboratory Summary				
Average	97.00 %		RSD	2.39 %

Table 6. Nevirapine				
Summary				
MUHAS			CAMAG	
Average	101.75 %		Average	96.08 %
rsd	1.39%		rsd	1.34 %
Interlaboratory Summary				
Average	98.92 %		RSD	3.21 %

Table 5. Lamivudine-Zidovudine				
MUHAS Summary			CAMAG Summary	
Lam average	104.4 %		Lam average	101.0 %
Lam rsd	2.58 %		Lam rsd	1.92 %
Zid average	106.8 %		Zid average	106.2 %
Zid rsd	2.05 %		Zid rsd	3.26 %
Interlaboratory Summary				
Lam average	102.7 %		Zid average	98.92 %
Lam RSD	2.88 %		Zid RSD	3.21 %

JAOAC publication

- E. Kaale, P. Risha, E. Reich, and T. Layloff. An Interlaboratory Investigation on the Use of High Performance Thin Layer Chromatography to Perform Assays of Lamivudine-Zidovudine, Metronidazole, Nevirapine, and Quinine Composite Samples, JOURNAL OF AOAC INTERNATIONAL VOL. 93, NO. 6, 2010

General Conclusion

Advantages of using HPTLC -Densitometry in Pharmaceutical analysis**:**
Our experience

- Similar precision and accuracy to HPLC
  - Interday and intraday
  - Sensitivity adequate for assay/DT/ID/Content Uniformity
- High sample throughput
  - Sample preparation involve few steps
  - 20-30 min 18 samples on one plate while it would be much longer for HPLC
- Relatively cheap
  - Solvents
    - Use less solvents 20 mL for 18 samples cf. more than 600 mL(30 min run 1 mL/min) in HPLC
    - Uses analytical grade not HPLC grade (cheap Methanol HPLC grade USD 82, analytical grade 39,) possibility of recycling
    - Reagents based on Minilab

Way-forward

- Set up inter laboratory collaborations for cross validation of the methods with key partners (SoP-TFDA, WHO, MSH, Camag)
  - **Proposal monograph to IP**
- Share the cross validated methods with local industries as means of reducing analytical costs and improving quality of products.

Acknowledgements

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