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Impact of Tropical Conditions on Thin Layer Chromatography in Analytical Toxicology: High Temperatures and Moderate Humidities

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ABSTRACT: The impact of high temperatures (24 to 39°C) and low to moderately high humidities (20 to 70%) on the applicability of TLC systems for drug identification was studied during a 6 month climatologic cycle in Burkina Faso (West Africa). In general, the Rf values as observed on the plates were found to be substantially affected as compared with values obtained at temperate climates. Some TLC systems were more affected than others and the largest deviations of up to 30 Rf units were at low humidities. Tropical conditions also had a negative effect on the reproducibility of Rf values. However, when an Rf-correction procedure was applied, using reference mixtures of known drugs on each plate, accuracy as well as reproducibility of the resulting Rf^c values were drastically improved and data thus corrected were found to be compatible with existing TLC data bases developed under moderate climatological conditions.

The impact of high to extremely high humidities (70 to 100%) remains to be investigated.

KEYWORDS: toxicology, TLC, screening, analytical toxicology, temperature, relative humidity, climatic conditions

Virtually all of the commonly used thin layer chromatographic (TLC) procedures for drug identification in analytical toxicology have been developed in the western world under moderate climatic conditions. Although it is known that TLC—as an open technique—can be affected by factors such as temperature and humidity [*1*], little or no information is available on how these procedures are affected by tropical conditions (for example, those prevailing between the tropics and characterized by temperatures usually above 25°C). The latter is of vital importance for many developing countries (simplicity and low costs), and in situations where climatized conditions are not available, field work for example.

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We have investigated the impact of high temperatures of 24 to 39°C and prevailing relative humidities of 20 to 72% (dry to moderately humid) as they occurred during the 6 month climatologic cycle (January through June) in Burkina Faso, West Africa, under routine laboratory circumstances. This was preferred over the use of climatized rooms since the latter cannot take into account changes during the day, draught, open doors and windows, etc.

As we were primarily interested in the applicability of TLC toward drug identification, we examined a number of established screening systems with regard to the reproducibility and the accuracy of the Rf values as observed on the plates and after applying a Rf correction procedure [2]. Accuracy was assessed by comparing the Rf-data bases generated under moderate climatic conditions [2,3]. In this article, Rf values are expressed as so-called hRf-values:

$$\text{hRf} = \frac{\text{distance the substance travels from the origin}}{\text{distance the solvent travels from the origin}} \times 100$$

Experimental

Selection of Test Drugs

Two groups of 30 drugs were selected from the WHO list of essential drugs:

Acidic and Neutral Drugs (A/N)

acetanilide	chlorthalidone	phenobarbital	sulfafurazole
acetazolamide	chlorazepate	phenytoin	sulfathiazole
acetylsalicylic acid	clonazepam	prazepam	temazepam
aprobarbital	diazepam	salicylamide	thiopental
benzocaine	hydrochlorothiazide	salicylic acid	tolbutamide
caffeine	ibuprofen	secobarbital	vinbarbital
carbimazole	paracetamol	sulfacetamide	
carbromal	phenacetin	sulfadimidine	

Basic and Neutral Drugs (B/N)

amitriptyline	codeine	morphine	promethazine
amphetamine	desipramine	neostigmine bromide	propranolol
atropine	diamorphine	nicotinamide	quinine
benzalkonium chloride	diazepam	papaverine	reserpine
chloramphenicol	hydroxyzine	pethidine	tetracaine
chlorprotixene	isoniazide	physostigmine	trimipramine
cinnarizine	lidocaine	pilocarpine	
cocaine	mebendazole	procaine	

TLC Systems

According to the recommendations of TIAFT [2], systems 1–4A were used for acidic and neutral drugs and systems 4B–10 for basic and neutral drugs:

	System	Error Window
1.	chloroform-acetone (80:20)	7
2.	ethyl acetate	5
3.	chloroform-methanol (90:10)	8

4A. ethyl acetate-methanol-conc. ammonia (85:10:5)	11
4B. ethyl acetate-methanol-conc. ammonia (85:10:5)	10
5. methanol	8
6. methanol-butanol (60:40), 0.1 mol/L NaBr	9
7. methanol-conc. ammonia (100:1.5)	7
8. cyclohexane-toluene-diethylamine (75:15:10)	7
9. chloroform-methanol (90:10)	11
10. acetone	9

The systems were run on TLC plates silica gel 60 F_{254} with fluorescence indicator (Merck, Darmstadt, Germany), for systems 7–10 impregnated with KOH [*2*]. Paper-lined, saturated tanks were used (presaturation 30 min), except for systems 5 and 6 which were run in unsaturated tanks. The error window for a given system is three times the standard deviation for that system, determined in interlaboratory studies. See reference 2.

B/N drugs were also evaluated on Toxi-Gram A plates (Toxi-Lab, Irvine, Cal.), following the Toxi-Lab procedure [*3*] and with ethyl acetate-methanol-water (87:3:1.5) as solvent to which 10 to 20 μL conc. ammonia was added.

Detection was done under UV light and by means of location reactions [*3*]. For each drug, Rf values were determined in 10 fold over a period of 6 months (January through June).

Rf-Correction Procedure

On each TLC plate a mixture of four reference substances was spotted and the Rf values observed were compared with their corresponding reference data base values (Ref *2* for systems 1–10, Ref *3* for the Toxi-Lab system), so that a six point correction graph was obtained for each experiment, including the starting point (0,0) and the solvent front (100,100). The observed Rf values of the test drugs in that experiment were then corrected by means of the graph or by calculation [*2*]. Figs. 1 and 2 depict typical correction graphs for systems 4B and 7, respectively.

The reference mixtures for the respective systems, with the Rf values in parentheses (so called Rf^c values as found in Refs *2* and *3*) were as follows: System 1: paracetamol (15), clonazepam (35), secobarbital (55), methylphenobarbital (70). System 2: sulfathiazole (20), phenacetin (38), salicylamide (55), secobarbital (68). System 3: hydrochlorothiazide (11), sulfafurazole (33), phenacetin (52), prazepam (72). System 4A: sulfadimidine (13), aprobarbital (36), temazepam (63), prazepam (81). System 4B: morphine (20), codeine (35), hydroxyzine (53), trimipramine (80). System 5: codeine (20), trimipramine (36), hydroxyzine (56), diazepam (82). System 6: codeine (22), diphenhydramine (48), quinine (65), diazepam (85). System 7: atropine (18), codeine (33), chlorprothixene (56), diazepam (75). System 8: codeine (6), desipramine (20), prazepam (36), trimipramine (62). System 9: desipramine (11), physostigmine (36), trimipramine (54), lidocaine (71). System 10: amitriptyline (15), procaine (30), papaverine (47), cinnarizine (65). Toxi-Lab: morphine (15), codeine (24), amphetamine (32), methadone (66). Solutions of the four reference substances in a suitable organic solvent contained approximately 2 mg/mL of each substance and were stored in the refrigerator. 1 to 2 μl was spotted.

Climatic Conditions

Temperature and relative humidity of each experiment was recorded when the plate was put in the tank for development. Temperatures varied from 24 to 39°C, and relative

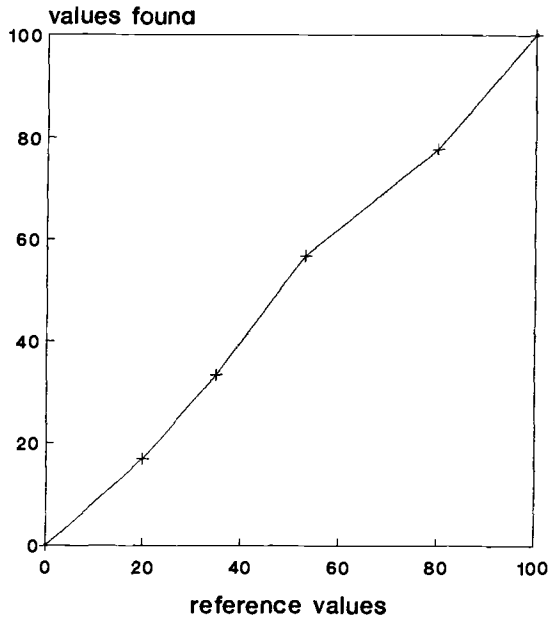

FIG. 1—*Typical Rf-correction graph for system 4B. Reference substances were (Rf^c values in brackets) morphine [20], codeine [35], hydroxyzine [53], trimipramine [80].*

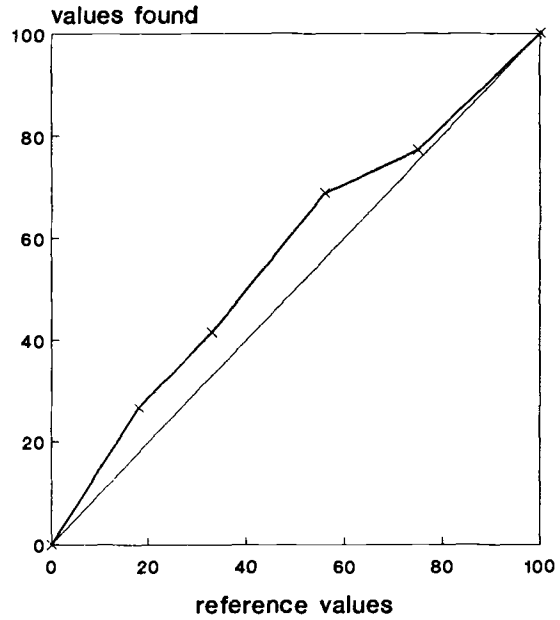

FIG. 2—*Typical Rf-correction graph for system 7. Reference substances were (Rf^c values in brackets) atropine [18], codeine [33], chlorprothixene [56], diazepam [75].*

humidities from 20 to 72%. The majority of the experiments was carried out between 28 to 35°C and 30 to 60% relative humidity.

Evaluations

Reproducibility was assessed as follows: for each substance in a given system the individual standard deviations around the mean (SD) were calculated. Then, these SDs were averaged over all substances investigated in that system to give $\overline{SD}$s. This was done for uncorrected Rf values as well as for corrected ones. The number of observations per substance was at least 10.

Accuracy was also assessed per system before and after correction of the Rf values. First, for each substance, the mean deviation (MD) between the observed Rf value and the one available in the literature was calculated:

$$MD = \frac{\Sigma\,(Rf_{observed} - Rf_{literature})}{n}$$

in which n represents the number of observations (at least 10) per substance. Then, these MDs were averaged over all substances investigated in that system to give the averaged mean deviation from the literature Rf values:

$$\overline{MD} = \frac{\Sigma\,MD}{m}$$

in which m represents the number of substances investigated. In addition, the mean *absolute* deviation (MAD) from the literature was calculated in a similar way:

$$MAD = \frac{\Sigma|Rf_{observed} - Rf_{literature}|}{n}$$

and for the averaged mean deviation from the literature:

$$\overline{MAD} = \frac{\Sigma\,MAD}{m}$$

$\overline{MAD}$ is the parameter of choice to assess accuracy because it considers deviations from the literature irrespective of sign. With $\overline{MD}$, deviations will level out if some substances run higher with others running lower than their literature values. As a result the $\overline{MD}$ may be close to zero, even though the deviations can be substantial.

Results and Discussion

All systems could be used under the climatic conditions encountered. No solvent demixing or irregular development was observed. It should be noted, however, that at higher temperatures (>32°C) the ammonia tended to 'boil' when the bottle was opened, that is, gas bubbles developed. When the ammonia was stored in bottles of 1 litre, this phenomenon resulted in large losses of ammonia and a considerable shift in the migration of the substances. Therefore, in later experiments, ammonia was stored in 100 mL bottles.

When the uncorrected Rf values were considered, it became clear that tropical conditions could cause drastic changes as compared to the data in the literature (accuracy) as well as bad reproducibilities ($\overline{SD}$s up to 5). This is summarized in Table 1 under the

TABLE 1—*Reproducibility and accuracy of Rf values.*

TLC system	$\overline{SD}$[a]		$\overline{MD}$[b]		$\overline{MAD}$[c]	
	U	C	U	C	U	C
1	3.2	2.4	−5.4	−1.4	6.2	3.2
2	3.1	2.8	−4.5	−1.1	5.1	3.0
3	4.7	2.6	−2.0	−1.7	2.9	2.5
4A	2.8	2.9	−3.1	1.6	5.0	4.1
4B	3.5	2.5	−1.4	−1.0	2.3	1.7
5	2.5	1.7	1.6	1.1	3.3	2.2
6	4.7	3.1	−2.8	0.7	5.3	3.2
7	3.2	3.1	10.4	2.4	10.5	3.0
8	3.3	2.7	2.0	1.0	2.5	1.6
9	4.3	2.5	5.8	1.5	6.3	3.6
10	2.8	1.9	5.3	−1.4	7.7	3.6
Toxi-Lab	2.5	0.7	−0.0	−0.3	2.5	1.7

[a]$\overline{SD}$ = averaged standard deviation of the mean per system.
[b]$\overline{MD}$ = averaged mean deviation from the literature; $Rf_{found} - Rf_{lit}$ per system.
[c]$\overline{MAD}$ = averaged mean absolute deviation from the literature; $|Rf_{found} - Rf_{lit}|$ per system.

U columns. The systems 1 to 4A tended to give too-low Rf values, whereas the systems 7 to 10 for basic and neutral drugs gave too-high Rf values. Yet, some systems were more affected than others. System 7 was the worst with deviations for individual substances sometimes around 30 Rf units! Other systems with low accuracies are 1, 2, 4A, 9, and 10. The better systems were 3, 4B, 5, 8, and Toxi-Lab. Uncorrected Rf values usually increased with humidity and temperature, with a few exceptions. Yet, the impact of the humidity was much more pronounced than that of the temperature. This is demonstrated in Table 2 for the best system, Toxi-Lab. Under rather dry conditions the uncorrected Rf values were substantially lower than the literature values, whereas at relatively high humidities almost all Rf values were above those in the literature. A notable exception is paracetamol. The other systems showed even larger deviations under these circumstances.

However, the Rf-correction procedure dramatically improved the applicability of all the systems under tropical conditions (Table 1). This is clearly reflected by the considerable reduction in $\overline{SD}$ (better precision), but even more so by the drastic reductions in deviations from the literature values ($\overline{MD}$s and $\overline{MAD}$s). Again, the better systems were 3, 4B, 5, 8, and Toxi-Lab. The relative value of $\overline{MD}$ is demonstrated by system 6 in

TABLE 2—*Impact of humidity on uncorrected Rf values obtained in the Toxi-Lab system.*

Substance	Rf[c] in lit.	Observed Rf and RH			Range
		Low 20–22%	Medium 30–60%	High 72%	
Diazepam	90	90	91–92	93	3
Cocaine	79	65	80–82	92	27
Paracetamol	75	80	75–78	71	9
Caffeine	64	56	60–62	66	10
Imipramine	50	30	49–54	68	38
Pethidine	50	34	48–52	68	34
Amphetamine	32	22	31–36	50	28
Methamphetamine	22	11	19–21	37	26

Temperature: 28 to 32°C.
RH = Relative Humidity.

Table 1, with a $\overline{MD}$ after correction of 0.7, versus a $\overline{MAD}$ of 3.2. Obviously there is considerable deviation of the observed corrected values from the ones in the literature, but some substances run higher and others lower than their literature values. On the other hand, the nearly identical $\overline{MD}$ and $\overline{MAD}$ values for system 7 before correction indicate that virtually all observed Rf values are much higher than the literature values.

When looking at the individual Rf^c values obtained after correction (300 per system), some still remained outside the error windows or search windows obtained previously under moderate climatic conditions (see experimental section). This was the case for systems 2, 7, and 8. Therefore, we recommend that for work under tropical conditions these error windows be increased to 8, 9, and 8, respectively. Another important observation was that the Rf-correction procedure was equally effective at high and low temperatures and humidities and that it maintained its utility even when climatic conditions changed rapidly, during tropical showers, for example.

Thus, Rf corrections using reference samples on the same plate appear to be essential for TLC work under tropical conditions and the corrected Rf^c values obtained in this way are compatible with the existing TLC data bases developed under moderate climatic conditions, at least for humidities up to 70%. The impact of higher humidities is being studied.

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