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Identification of drugs in autopsy liver samples by instrumental qualitative thin-layer chromatography

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Abstract

An instrumental thin-layer chromatographic (TLC) procedure is described for the identification of drugs with use of corrected R_F values (hR_F^c) and *in situ* ultraviolet spectra. One hundred and eleven successive autopsy liver samples received from medical examiners were investigated by this technique and the results were compared to those obtained with a reference method. From the nineteen findings by the reference method, sixteen (84%) were correctly identified by the instrumental TLC method, using an hR_F^c pre-search with a window size of +/−7 units followed by a correlation search. In addition, one drug was identified correctly by correlation search only. There was no serious interference from endogenous substances, and a correlation value of about 0.9 is suggested as a limit to cut the hit list of candidates.

1. Introduction

Thin-layer chromatography (TLC) belongs to the basic method arsenal of forensic drug analysts, and it has been stated that TLC should be the first test in any drug analysis protocol [1]. During the last decade, the development of instrumental TLC [2] has created new possibilities for the quantitative analysis of drugs. However, qualitative TLC still largely relies on manual methods which, although often efficient, are usually too much dependent on individual analysts' skills in interpreting the chromatograms.

Recently, a new concept for qualitative analysis by instrumental TLC was reported: correction of R_F values by the polygonal method with several standards, and searching libraries using the corrected R_F values (hR_F^c) and UV spectra. A study involving five laboratories proved that drug libraries consisting of hR_F^c values and UV spectra can be used for identification on an interlaboratory basis, and promising results were obtained in the analysis of selected biological samples for drugs [3].

In the present study, instrumental qualitative TLC is evaluated for the identification of basic drugs in autopsy cases. A series of one hundred and eleven successive liver samples received from medical examiners were analysed during an eight-day period, and the results were compared with those obtained with a reference method.

2. Experimental

2.1. Chemicals

Trypsin of type IX was purchased from Sigma (St. Louis, MO, USA). Bis(2-ethylhexyl)-

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hydrogen phosphate (HDEHP) was from Aldrich (Steinheim, Germany).

2.2. Chromatography

The TLC plates were 10×20 cm glass plates precoated with 0.25 mm Silica Gel 60 F_{254} (Merck, Darmstadt, Germany). The plates were developed to a distance of 7 cm in an automatic developing chamber (ADC) (CAMAG, Muttenz, Switzerland). The mobile phase (12 ml) was toluene–acetone–94% ethanol–25% ammonia (45:45:7:3, v/v) [4], and presaturation (1 min) was carried out with the mobile phase without ammonia.

2.3. Densitometric evaluation

The scanning densitometer was a TLC Scanner II operated with CATS 3.16 software (CAMAG). The plates were scanned by absorbance at 220 nm, the spots were integrated and the spectra were recorded in the range of 200–400 nm with 5 nm wavelength increments. The R_F values were corrected by the polygonal method [5] using four reference compounds [6]: codeine ($hR_F^c = 16$), promazine (36), clomipramine (49) and cocaine (66).

2.4. Drug library

A library of 100 basic and quaternary drugs was created by chromatographing pure drug standards (4 μg) using the mobile phase described above with a saturated twin-trough chamber. The hR_F^c values were taken from existing data [4].

2.5. Sample preparation

The enzyme digestion and ion-pair extraction procedure for liver samples is shown in Fig. 1.

2.6. Reference method

The reference method for the analysis of liver samples was a combination of the present TLC system and an RP-18 system [4] using several post-chromatographic visualization reagents. Normal phase chromatography was carried out on three plates to allow undisturbed use of various reagents. The liver findings were supported by blood analysis by dual-column capillary gas chromatography with nitrogen specific or electron-capture detection.

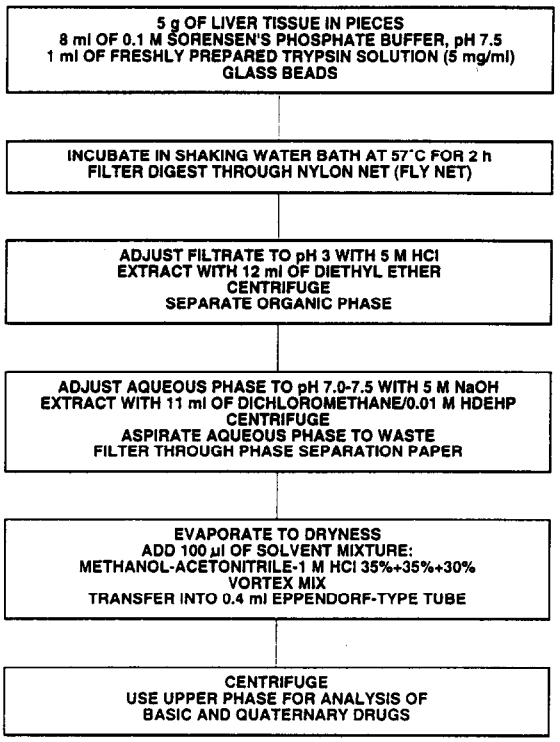

Fig. 1. Extraction procedure of liver samples for TLC.

3. Results and discussion

3.1. Description of the spectrum library option

The following example shows the function of the spectrum library option of CATS software. Thirteen autopsy liver extracts are run parallel with a set of four correction standards on a separate track (Fig. 2). The user assigns the standards on the monitor screen by clicking with the mouse. The programme calculates the hR_F^c

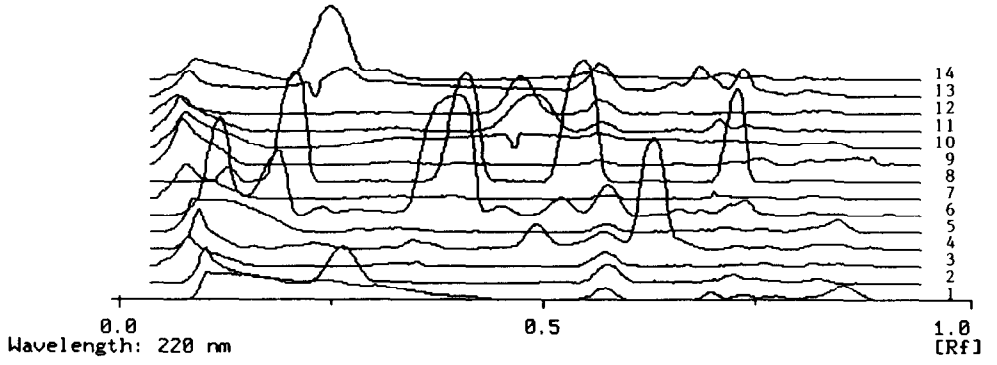

Fig. 2. Thirteen liver extracts chromatographed parallel with a set of four correction standards on track 8.

values of the unknowns based on the standards, using the origin and the solvent front as additional correction points [5]. The hR_f^c values are then listed together with track numbers, possible substance names and UV maximum wavelengths.

To identify an intensive spot on track 6 at hR_F^c 35, the respective position in the list is clicked and the spectrum is displayed. By using an hR_F^c pre-search with a window size of $+/-7$ hR_F^c units prior to the correlation search, a hist list is obtained that ranks promazine first with a correlation value of 0.998. Superimposing the sample spectrum with the best library match shows considerable similarity (Fig. 3).

3.2. Systematic analysis of liver samples

One hundred and eleven successive autopsy liver samples received from medical examiners were analysed for basic and quaternary drugs by the instrumental TLC method and by the reference method. There were nineteen findings by the reference method for such drugs that were included in the present drug library of one hundred compounds (Table 1). In addition, carbamazepine, citalopram, moclobemide, oxazepam, pentobarbital, phenytoin, salicylate, temazepam and triamterene, not included in the present library, were found once or more often by the reference method.

Table 1 shows the library search results obtained by the instrumental TLC method for each of the nineteen findings. The average correlation value of the sample spectrum with the correct library spectrum was 0.95 (S.D. 0.08). The correct drug was ranked first in sixteen cases out of nineteen (84%) by the combination hR_F^c presearch and correlation search. In addition, in one case (3138/93) the drug was out of the hR_F^c search window because of its very high concentration and partial co-eluting with a metabolite but was ranked first by the correlation search only.

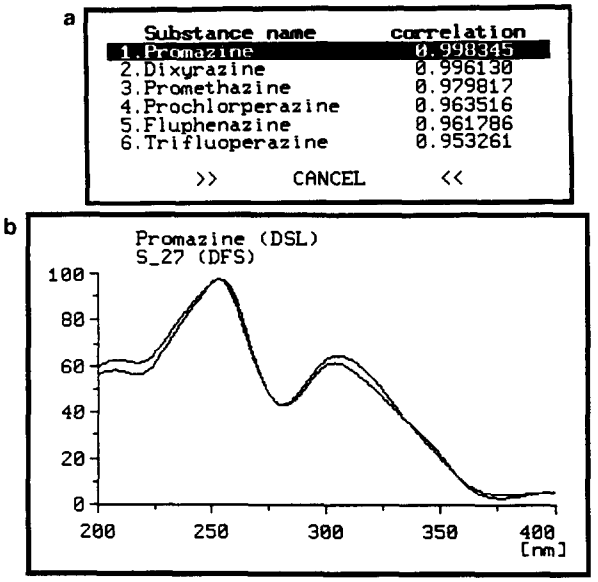

Fig. 3. Library search hit list obtained by hR_F^c + correlation for the spot at hR_F^c 35 on track 6 (a), and the best match superimposed with the sample spectrum (b).

Table 1
Drug findings from 111 successive autopsy liver samples received from medical examiners

Drug	Case No.	hR_F^c found	hR_F^c libr.	Correl. value	Hit list position[a]	Blood conc. (mg/l)
Levomepromazine	3031/93	56	60	0.983	1st	2.9
Clomipramine	3032/93	42	49	0.990	1st	30
Thioridazine	3035/93	43	45	0.917	1st	0.5
Promazine	3037/93	35	36	0.998	1st	13
Amitriptyline	3052/93	40	46	0.937	1st	0.8
Promazine	3053/93	34	36	0.976	3rd	4.0
Trimipramine	3053/93	65	65	0.651	–	0.6
Dextropropoxyphene	3067/93	64	67	0.990	1st	2.9
Promethazine	3102/93	46	41	0.979	1st	3.4
Dextropropoxyphene	3104/93	66	67	0.993	1st	0.2
Levomepromazine	3108/93	60	60	0.995	1st	1.0
Clomipramine	3122/93	46	49	0.963	1st	4.5
Levomepromazine	3122/93	60	60	0.941	1st	2.9
Chlorprothixene	3123/93	58	56	0.998	1st	1.2
Amitriptyline	3136/93	44	46	0.963	1st	2.0
Nortriptyline	3136/93	19	20	0.948	1st	–
Trazodone	3138/93	64	53	0.995	1st[b]	23
Nortriptyline	3149/93	20	20	0.960	1st	0.7[c]
Thioridazine	3149/93	45	45	0.953	1st	4.1

[a] Hit list generated by hR_F^c pre-search (+/−7 units) followed by correlation search.
[b] Library search by correlation only.
[c] Concentration for amitriptyline.

Table 2 shows the best library matches and the corresponding spectrum correlations for all spots in thirteen successive liver samples. There were only few endogenous substances that could cause false interpretation. One such compound was observed at hR_F^c 20–22 in some cases (tracks 2 and 14), giving a metoprolol-like spectrum. Inclusion of this compound or other interfering compounds in the drug library would greatly facilitate the interpretation of the results. Investigation of all the 111 cases of this study revealed that a correlation value of about 0.9 could be used as a limit to cut the hit list of candidates.

Drug metabolites often produce UV spectra very similar to that of the parent drug. This can be seen on track six where promazine and its metabolite give UV spectra that resemble each other (Fig. 4). Inclusion of the most important metabolites in the drug library would prevent misinterpretations and aid the identification of at least antipsychotic and antidepressant drugs.

3.3. Requirements for comprehensive drug screening

No single TLC method, not even an instrumental one, can positively identify all the hundreds of drugs available. However, if a second TLC system with a low correlation is added to the first one, the situation is better. The combination of an RP-18 reversed-phase system with the present TLC system has proved to be very efficient in the general screening of basic and quaternary drugs [4,6,7]. One of the most characteristic features of TLC is the possibility to utilize post-chromatographic off-line derivatization. The many visualization reactions available provide a means for further confirmation of findings that, *e.g.*, high-performance liquid chromatography is lacking.

Post-chromatographic visualization reactions can be utilized satisfactorily even if the separation of analytes is not optimal. *In situ* UV spectra are more disturbed by co-eluting of

Table 2
Best library matches obtained for all spots of the 13 successive liver samples of Fig. 2

Track No.	Case No.	hR_F^c found	Best library match[a]	Correl. value
1	2979/93	8	Maprotiline	0.875
1		52	Clomipramine	0.789
1		81	Meclozine	0.666
2	3033/93	8	Maprotiline	0.705
2		22	Oxprenolol	0.978
2		52	Clomipramine	0.788
3	3034/93	6	Maprotiline	0.797
3		51	Clomipramine	0.716
4	3035/93	8	Maprotiline	0.862
4		43	Thioridazine	0.917[b]
4		51	Clomipramine	0.801
4		57	Pitofenone	0.921
5	3036/93	7	Maprotiline	0.848
5		8	Maprotiline	0.870
5		81	Meclozine	0.644
6	3037/93	9	Acebutolol	0.848
6		14	Opipramol	0.900
6		35	Promazine	0.998[c]
6		46	Promethazine	0.900
6		52	Clomipramine	0.806
6		67	Levomepromazine	0.812
7	3038/93	6	Maprotiline	0.593
8[d]				
9	3039/93	6	Maprotiline	0.851
10	3040/93	0	Maprotiline	0.756
10		40	No match	
10		42	No match	
11	3041/93	5	Maprotiline	0.830
11		43	Amitriptyline	0.886
12	3042/93	5	No match	
12		42	Periciazine	0.894
12		51	Clomipramine	0.664
13	3043/93	7	No match	
13		17	No match	
13		22	Pindolol	0.799
13		51	Clomipramine	0.797
13		62	Pentazocine	0.842
13		67	Levomepromazine	0.896
14	3044/93	7	Maprotiline	0.774
14		20	Metoprolol	0.987

[a] Hit list generated by hR_F^c pre-search (+/−7 units) followed by correlation search.
[b] Correct result (see Table 1, case 3035/93).
[c] Correct result (see Table 1, case 3037/93).
[d] Standard track.

substances, and consequently the use of high-performance thin-layer chromatography (HPTLC) plates to improve resolution may prove to be feasible. However, to avoid over-loading in HPTLC a scale-down is probably necessary, and further studies are needed to estimate the applicability of spectrum libraries at the lower concentration range.

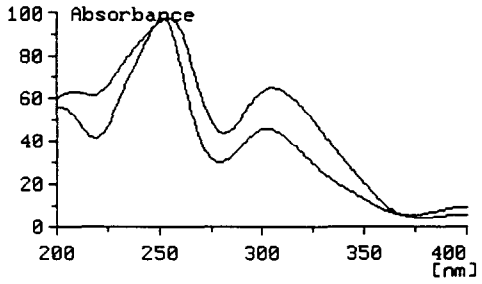

Fig. 4. Spectra of promazine and its metabolite superimposed.

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