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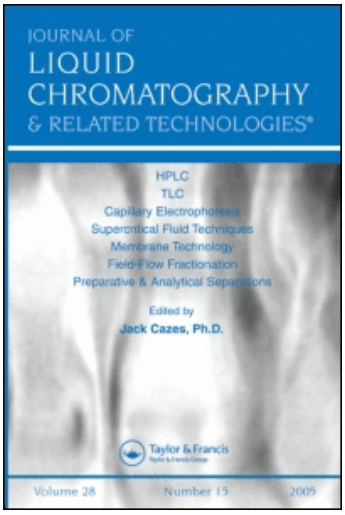

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Ilkka Ojanperä[a]; Erkki Vuori[a]
[a] Department of Forensic, Medicine University of Helsinki Kytosuontie, Helsinki, Finland

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THIN LAYER CHROMATOGRAPHIC ANALYSIS OF BASIC AND QUATERNARY DRUGS EXTRACTED AS BIS(2-ETHYLEXYL)PHOSPHATE ION-PAIRS

Ilkka Ojanperä and Erkki Vuori
Department of Forensic Medicine
University of Helsinki
Kytosuontie 11
SF-00280 Helsinki, Finland

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ABSTRACT

Thin layer chromatography in the normal and reversed phase mode is evaluated as a qualitative analysis method for various basic and quaternary drugs extracted as bis(2-ethylhexyl)phosphate ion-pairs. A combination of two thin layer chromatographic systems, a reversed phase system and a fairly apolar normal phase system, is shown to cover the analysis of drugs with different grades of polarity. Based on that, a method for screening of urine samples for drugs is described.

INTRODUCTION

Ion-pair formation has been frequently utilized for both enhancing the extraction recoveries of hydrophilic ionizable compounds, and in adjusting retention behaviour in liquid chromatography. Ion-pair methods have proven particularly effective in drug analysis, for pure substances, pharmaceutical preparations, and biological material (1,2).

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Bis(2-ethylhexyl)phosphoric acid (HDEHP) is a counter-ion with high extraction efficiency, due to its hydrophobicity and adduct-forming ability (3). Drugs extracted as HDEHP ion-pairs have been analysed mainly by UV spectrophotometry (3-5), but also by gas chromatography after derivatization (6). A method involving HDEHP extraction and high performance liquid chromatographic analysis of the extracts has been put forward as a standardized analysis strategy for basic drugs (7).

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For drug screening purposes, thin layer chromatography (TLC) is often superior to other methods in terms of versatility and speed. In this study, TLC in the normal and reversed phase (RP) mode was evaluated as a qualitative analysis method for various basic and quaternary drugs extracted from buffer solution as HDEHP ion-pairs. The selected TLC systems were applied to the screening of urine samples for drugs.

EXPERIMENTAL

Standards: 3 μg (3 μl 1 mg/ml methanolic solution) of the drugs was applied to a plate.

HDEHP extracts: 10 ml of 0.1 M Sørensen's phosphate buffer, pH 7.4, with a drug concentration of 0.05 mg/ml was extracted for 15 minutes with 10 ml of dichloromethane

containing 0.01 M HDEHP. After centrifugation, 5 ml of the organic phase was evaporated to dryness, the residue was reconstituted with 250 µl of methanol, and 3 µl was applied to a plate.

Conventional extracts: The procedure was as for HDEHP extracts, but the drugs were extracted at pH 11.5 (the phosphate buffer was alkalized with conc. NaOH) to dichloromethane.

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Urine extracts: 5 ml of post-mortem urine was acidified to pH 2-3 with dilute HCl, it was extracted for 10 minutes with 10 ml of diethyl ether, and the mixture was centrifuged. The separated aqueous phase was adjusted to pH 7 with dilute NaOH and with 2 ml of 0.1 M Sorensen's phosphate buffer, pH 7.0, and it was extracted for 15 minutes with 10 ml of dichloromethane containing 0.01 M HDEHP. After centrifugation, the separated dichloromethane phase was evaporated to dryness, and the residue was shaken with 100 µl of a mixture being composed of 70 % of methanol and 30 % of 1 M HCl. After centrifugation, 5 µl of the supernatant was applied to three silica gel plates and to one RP plate.

Thin layer plates: TLC precoated plates Silica Gel 60 F_{254} (No. 5554) and RP-18 F_{254}s (No. 15423) from Merck were used in the size of 10 cm x 20 cm. The RP plates were dryed according to manufacturer's directions prior to use.

Development: The plates were developed for 7 cm in a double trough developing tank from Camag, using eight solvent systems for test drug extracts and two of them (systems II and VIII) for urine extracts (see Results).

Detection: For examination of the test drug chromatograms, the plates were first viewed under 254 nm and 366 nm UV light. After that, they were sprayd with acidified iodoplatinate solution (8) (mixed with 0.5 parts of ethanol for RP-18 plates) for the quaternary ammonium compounds and the antipsychotic drugs, and with 0.5 % aqueous solution of Fast Black K salt followed by 0.5 M NaOH (9) for the others. The detection of urine extracts was performed according to the following practice (8,9): Plate 1 (silica): UV, FPN reagent, Marquis reagent. Plate 2 (silica): UV, Dragendorff reagent, acidified iodoplatinate reagent. Plate 3 (silica): UV, Fast Black K salt reagent. Plate 4 (RP): UV, Fast Black K salt reagent, acidified iodoplatinate reagent. Occasionally an extra RP plate was treated with Marquis reagent.

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RESULTS AND DISCUSSION

The nineteen test drugs, which were amines and quaternary ammonium compounds, represent six therapeutic categories and different grades of polarity. Some of the drugs were rather hydrophilic compounds, which were not extractable conventionally from strongly basic aqueous

solution to dichloromethane (Table I). However, all of the drugs could be extracted as HDEHP ion-pairs.

In contrast with most other counter-ions that have been used in ion-pair extraction, the excess of HDEHP remains in the organic phase, and it is concentrated in the evaporation residue. Because attempts to separate HDEHP from the drugs before TLC analysis always lower the recovery of certain drug groups, the TLC system should be able to separate the ion-pair.

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The chromatographic behaviour of the drugs extracted as HDEHP ion-pairs was compared with drug standards in seven widely used normal phase TLC systems (10) (Table II). In the moderately apolar basic systems, I and II, the R_f values and the spot shapes of the less polar extracted drugs were identical with the standards. However, the HDEHP spot interfered with the more polar drugs, up to hR_f 5 in the system I and up to hR_f 18 in the system II. In the polar basic system, III, many of the extracted drugs were distorted by HDEHP, which caused a strongly tailing spot in the upper region. The rest of the extracted drugs produced rather badly defined spots in this system.

The neutral systems, IV, V, VI and VII, differed from each other in their ability to dissociate the ion-pair. In the fairly apolar system, IV, the distribution of the drugs resembled systems I and II, HDEHP causing interference up to

TABLE I

Test Drugs and their Properties

Drug	Main chemical class related to ionizability	Conventional extraction behaviour[a]
Anticholinergic drugs		
1. Butylscopolamine Br	Quaternary ammonium compound	--
2. Emepronium Br	Quaternary ammonium compound	--
Sympathomimetic drugs		
3. Ephedrine HCl	Sec. amine	
4. Isoprenaline 1/2 H_2SO_4	Sec. amine, catechol	--
5. Salbutamol	Sec. amine, phenol	--
6. Terbutaline 1/2 H_2SO_4	Sec. amine, resorcinol	--
Beta adrenergic blocking drugs		
7. Acebutolol HCl	Sec. amine	
8. Atenolol	Sec. amine	-
9. Metoprolol HCl	Sec. amine	
10. Sotalol HCl	Sec. amine, sulfonamide	--
Neuromuscular blocking drugs		
11. Tubocurarine Cl	Quaternary ammonium compound	--
Antipsychotic drugs		
12. Chlorprothixene	Tert. amine	
13. Haloperidol	Tert. amine	
14. Perphenazine	Tert. amine	
15. Sulpiride	Tert. amine, sulfonamide	-
Opioid and opioid antagonist drugs		
16. Buprenorphine HCl	Tert. amine, phenol	
17. Morphine HCl	Tert. amine, phenol	--
18. Nalorphine HBr	Tert. amine, phenol	--
19. Pentazocine HCl	Tert. amine, phenol	

[a] - = Considerably lower recovery in conventional extraction compared with HDEHP extraction, spots clearly smaller
-- = Very low recovery in conventional extraction, no spots detected

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TABLE II

hR_f Values of Test Drugs Extracted with HDEHP

Drug	Normal phase systems							Reversed phase system
	I	II	III	IV	V	VI	VII	VIII
1	a	a	a	a	a	35	a	22
2	a	a	a	a	a	28	a	7
3	a	21	29	a	a	69c	b	51
4	a	b	b	a	a	75	b	80
5	a	b	50c	a	a	78	b	72
6	a	19c	53c	a	a	77c	b	73
7	a	24	49	a	a	72c	b	29
8	a	b	46	a	a	63c	b	67
9	10	34	53	b	7c	77	b	31
10	a	22	56	a	a	75c	b	71
11	a	a	a	a	a	12	a	50
12	53	66	59c	47	26c	49	38	a
13	12	66	b	25	32c	75c	b	7c
14	7	34	62c	26	8c	41	42c	b
15	a	26	47	b	11c	25	17c	67
16	8	71	b	67	65	b	76c	10
17	a	b	37	b	a	25c	20c	73
18	a	27	b	19	15c	59	63c	64
19	15	63	b	18	33c	78c	b	19

a = $hR_f < 5$
b = shapeless spot, or R_f value differs much from the standard
c = bad spot shape

I Cyclohexane-Toluene-Diethylamine 75:15:10 (Ref. 11)
II Ethyl acetate-Methanol-NH_3 85:10:5 (Ref. 10)
III Methanol-NH_3 100:1,5 (Ref. 11)
IV Chloroform-Methanol 90:10 (Ref. 11)
V Acetone (Ref. 11)
VI Methanol-Butanol 60:40, 0,1 M NaBr (Ref. 12)
VII Methanol (Ref. 12)
VIII Methanol-Water-HCl 50:50:1
In the systems I, III, IV and V, plates impregnated with 0,1 M KOH and saturated chambers were used. In the system II, saturated chamber was used.

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hR_f 15. In system V, most of the extracted drugs produced bad spots, although HDEHP seemed to remain at the origin. In the ion-pair system, VI, all the drugs migrated, but the spot shapes were distorted by a tailing HDEHP spot in the upper region. System VII failed to separate the ion-pair in most cases, and many of the extracted drugs produced a tailing spot together with HDEHP at hR_f 80.

To improve TLC analysis of polar drugs extracted as HDEHP ion-pairs, a simple and reproducible RP ion-pair TLC system, VIII, was constructed (Table II). The system produced well shaped spots, and made it possible to analyse all but the most apolar drugs satisfactorily. Nearly all of the interference of HDEHP was eliminated, because the counter-ion remained at the origin. There was not any significant difference in the chromatographic behaviour between the extracted drugs and the standards. By increasing the relative amount of methanol in the eluent, the R_f values can be raised, but this also results in a slight migration of HDEHP. An increase in the relative amount of water degrades the shape of the spots.

The use of the RP system makes it possible to utilize the high extracting power of HDEHP in the analysis of cationic organic compounds. An apolar normal phase TLC system and the RP-TLC system described here form an efficient complementary pair for analysing a wide range of basic and quaternary drugs extracted as HDEHP ion-pairs.

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This approach has proven useful in the toxicological screening of urine samples for drugs and poisons. After a traditional diethyl ether extraction of acidic and neutral drugs, cationic drugs are extracted as HDEHP ion-pairs to dichloromethane and separated using TLC systems II and VIII. By applying the standard selection of location reagents (8), supplemented with Fast Black K salt (9), the most polar drugs and many metabolites can be detected with system VIII, the most apolar drugs with system II, and medium polar drugs with both systems. Many drugs that were previously difficult to include in a TLC drug screen, e.g. morphine, terbutaline, sotalol and atenolol, have been detected and identified without problems using this general screening method.

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