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Note

Chromatographic detection of narcotic antagonists in human urine

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The detection and quantitation in urine of various drugs of abuse have been reported by a number of investigators[1–3]. The most popular detection system has been thin-layer chromatography (TLC) whereas gas–liquid chromatography (GLC) has been primarily utilized for quantitation of abused drugs in urine. Among the drugs studied have been the amphetamines, barbiturates, narcotics and analgesics[1–4]. However, little or no information is available concerning the TLC detection and GLC quantitation of narcotic antagonists in human urine in relationship to commonly abused drugs.

In this preliminary study, narcotic antagonists were detected in human urine utilizing the two most common analytical systems, TLC and GLC. In addition, antagonists were compared with a few commonly abused drugs which gave similar analytical results.

MATERIALS AND METHODS

For both the TLC and GLC procedures, the various standards were dissolved in chloroform or distilled water at a concentration of 1 mg/ml.

TLC procedure

Spiked urines were of pH 5–6 and extracted with either XAD-2 resin (Bio-Rad Labs., Rockville Centre, N.Y., U.S.A.) utilizing the Bio-Rad system[5] or a modification of the ion-exchange paper (SA-2, Analtech, Newark, Del., U.S.A.) system of Dole *et al.*[1]. TLC was carried out on a silica gel thin layer (Analtech). The solvent system utilized was ethyl acetate–methanol–ammonium hydroxide (17:2:1). Detection of the compounds was accomplished by utilizing a multiple spray system[2].

GLC procedure

Extraction of either spiked urines or patient urines was accomplished in the following manner: Urine (10 ml) was brought to pH 10.0 with 0.1 *N* NaOH. Chloro-

form was added and the mixture shaken for 10 min. The chloroform was evaporated to dryness at 60° on a water-bath with a stream of air blowing over the liquid. The dry residue was reconstituted with 100 μl of chloroform.

GLC was carried out on a Glowall (Willow Grove, Pa., U.S.A.), Model 310 instrument, equipped with a flame ionization detector. The chromatographic conditions were as follows: The temperatures of the injection block, the oven and the detector were 285°, 270° and 285°, respectively; the carrier gas was nitrogen at a flow-rate of 40 ml/min. The column employed was a 3% OV-17 on Chromosorb W-HP, 80–100 mesh (Supelco, Bellefonte, Pa., U.S.A.). One microgram of each of the samples was injected into the column. Preparation of the bis(trimethylsilyl)acetamide (BSA) derivatives was carried out according to the method of Weinstein *et al.*[6].

RESULTS AND DISCUSSION

Table I illustrates the results of the extraction and detection of narcotic antagonists in spiked human urine by two common drug abuse screening methods. In both systems, the minimal concentration of narcotic antagonists detected was 25 μg/25 ml or 1 μg/ml. At a lower concentration of 10 μg/25 ml or 0.4 μg/ml narcotic antagonist was not detectable in either system. The R_F value for cyclazocine was calculated to be 0.86 which was very close to the R_F value of methadone, 0.92. However, the two drugs could be easily distinguished by detection sprays. The R_F values of both naltrexone and naloxone were calculated to be 0.64. However, owing to the close structural similarity of naltrexone and naloxone, separation of these antagonists was not accomplished with this system.

Table II illustrates the results of the GLC separation of narcotic antagonists and a few commonly abused drugs. The commonly abused drugs selected were methadone and morphine. They were selected owing to their close relative retention times

TABLE I

EXTRACTION AND DETECTION OF NARCOTIC ANTAGONISTS FROM SPIKED HUMAN URINE

Detection: D_1 = Ninhydrin, 0.1% acetone; D_2 = UV light; D_3 = diphenylcarbazone; D_4 = mercuric sulfate; D_5 = heat; D_6 = iodoplatinate; D_7 = Dragendorff's reagent. Color code: O = Orange; Pu = purple; B = blue; RPu = reddish purple; BPu = bluish purple.

Drug	*Extraction procedure*	$R_F \times 100$	*Detection*						
			D_1	D_2	D_3	D_4	D_5	D_6	D_7
Cyclazocine	Ion exchange	86	—	—	O	Pu	—	Pu	RPu
	XAD-2 resin	86	—	—	O	Pu	—	Pu	RPu
Naltrexone	Ion exchange	64	—	—	—	—	—	Pu	RPu
	XAD-2 resin	64	—	—	—	—	—	Pu	RPu
Naloxone	Ion exchange	64	—	—	—	—	—	Pu	RPu
	XAD-2 resin	64	—	—	—	—	—	Pu	RPu
Morphine	Ion exchange	39	—	B	—	—	—	B	BPu
	XAD-2 resin	39	—	B	—	—	—	B	BPu
Methadone	Ion exchange	92	—	—	—	—	—	O	O
	XAD-2 resin	92	—	—	—	—	—	O	O

TABLE II

GLC SEPARATION OF NARCOTIC ANTAGONISTS

Optimum conditions as given in Materials and methods.

Drug	*Relative retention times**	
	Free form	*BSA derivative*
Methadone	1.0	1.0
Cyclazocine	1.5	1.2
Naloxone	ND	6.8
Naltrexone	ND	10.6
Morphine	ND	2.7

* Retention times are relative to methadone (1.5 min for both free and BSA derivatives). ND = non-detectable under these conditions.

to cyclazocine under these chromatographic conditions. The results indicate that while the free forms of cyclazocine and methadone could be easily distinguished, the free forms of naloxone, naltrexone and morphine were not detectable. However, when BSA derivatives were formed, the separation and detection of all the above drugs were clearly demonstrated.

Table III illustrates the recoveries of narcotic antagonists from alkaline human urine utilizing chloroform as the extraction solvent. Three concentrations of narcotic antagonists (25, 50 and 100 μg per 10 ml of urine) were extracted with chloroform. The extracts were then quantitated utilizing GLC. The average recoveries of each drug were determined by at least two to four individual experiments. The recoveries of cyclazocine ranged from 80–100%, depending on concentration. The recoveries of naltrexone ranged from 97–100%. Naloxone recoveries ranged from 62–78% depending on concentration. Subsequently, the recoveries for cyclazocine and naltrexone have been established to be good to excellent under these conditions. However, the naloxone recoveries are somewhat poor considering the above two drugs.

Table IV illustrates the detection of narcotic antagonists in urine of patients receiving daily oral doses of 5 mg of cyclazocine or 50 mg of naltrexone. All urines

TABLE III

RECOVERIES OF NARCOTIC ANTAGONISTS EXTRACTED FROM ALKALINE HUMAN URINE

Quantitation by gas–liquid chromatography. See Materials and methods for optimum conditions.

Drug	*Urine concentration spiked (μg/10 ml)*	*Average urine concentration recovered (μg/10 ml)*	*Average % recovered*
Cyclazocine	25	20.7	82.6
	50	49.0	97.9
	100	97.0	97.0
Naloxone	25	15.6	62.5
	50	36.8	73.5
Naltrexone	25	24.8	99.3
	50	49.6	99.1
	100	100	100

TABLE IV

DETECTION OF CYCLAZOCINE AND NALTREXONE IN URINE OF TREATED PATIENTS

Optimum conditions as given in Materials and methods.

Drug	*Patient*	*Daily oral dose (mg)*	*TLC* $R_F \times 100$	*GLC* R_t *(min)*
Cyclazocine	1	5	86	1.8
	2	5	86	1.8
	Standard	—	86	1.8
Naltrexone	1	50	61	16
	2	50	61	16
	Standard	—	61	16

were collected within 20–24 h after the last oral dose. The urines were screened by the previous TLC method. All patients gave a positive result of cyclazocine or naltrexone, depending on the drug administered. The results were confirmed by GLC to be accurate.

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