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Identification of drugs of abuse in urine

I. A study of the Dole technique

DOLE *et al.*[1] developed a thin-layer chromatographic method for the detection in urine of narcotic drugs, quinine, barbiturates, amphetamines and some tranquilizers. The drugs are first absorbed on ion-exchange paper and then extracted at controlled pH values into an organic phase. An aliquot of the organic phase is concentrated and chromatographed. A series of spray reagents were developed to provide detection and confirmation. This report describes a study of the DOLE technique.

Experimental procedure

The urine samples used in this study were selected at random from the samples routinely received for analysis from the NIMH methadone treatment out-patient

program in New Orleans. The procedure as described by DOLE *et al.*[1] has been used except for the following changes.

Using three separate 50-ml portions of a urine sample, one ion-exchange paper was prepared for narcotic analysis, one for barbiturates and one for amphetamines detection. The drugs were then re-extracted from the resin paper and chromatographed. According to DOLE[2], the pH 2.2 re-extraction (for barbiturates) can be omitted in the monitoring of urine for alkaloids (pH 9.3 extraction). The pH 11.0 re-extraction (for amphetamines) was also run in these experiments without prior desorption from the paper of barbiturates and alkaloids.

Results and discussion

Limits of detection. Table I shows the approximate minimum amount of authentic drugs which could be detected in 50 ml of urine using the DOLE technique. These data were obtained by adding graded amounts of the drugs to 50 ml of urine until a positive test was just discernable. Although relatively small amounts of morphine, codeine, and quinine can be detected in a 50-ml sample, significantly larger amounts of methadone, phenobarbital and *d*-amphetamine are required for a positive test.

TABLE I

REALISTIC LIMITS OF DETECTION OF NARCOTICS, BARBITURATES, AND AMPHETAMINES IN 50 C.C. OF URINE OBTAINED WITH THE DOLE METHOD

Drug	*Approximate minimum amount of authentic standards of drugs which could be detected in 50 c.c. urine (mg)*
Morphine	0.02
Codeine	0.02
Quinine	0.005
Methadone	0.3
Phenobarbital	0.2
d-Amphetamine	0.4

The recovery of these latter three drugs from the urine is so poor that the accuracy of the technique is undoubtedly questionable because the analyst will obtain negative tests even though microgram quantities of these drugs are present in the urine sample. As a result "false" negative tests will be accumulated.

Although the above data is in disagreement with that of DOLE *et al.*[1] (which implies that about 1 μg of each of these drugs can be detected in 50 ml urine) the data of other researchers in the field support our findings. MULE[3] has recently reported limits of detection in close agreement with our data. HEATON AND BLUMBERG[4] observed that large amounts of amphetamine are detectable with the DOLE technique, but smaller amounts produced negative results. MARTIN[5] has reported that the DOLE procedure is missing "positive tests". Other workers[6] have discontinued analysis for barbiturates and amphetamines in the urine, because so few positive tests could be obtained, even after running several hundred samples.

Duplicate tests. MULE[3] and HEATON AND BLUMBERG[4] showed that the poor sensitivity of the DOLE technique for methadone, barbiturates and amphetamines was

due to the fact that the ion-exchange paper (SA-2) did not release the drugs in the re-extraction steps. HEATON AND BLUMBERG[4] stated that the release of amphetamines from the resin paper proved to be erratic. MULE[7] commented that there was some variability in drug releases with different batches of resin paper.

Differences in the efficiency of the resin paper would be expected to adversely affect not only the accuracy of the technique but also the precision of the method. The problem becomes acute when one realizes that in those urine samples where the drug concentration is just high enough for detection, the technique is being operated at its limit of detection. In other words, one would predict that differences in resin paper efficiency are very important at low drug levels but should not be as important at high levels.

The data in Table II show the results of duplicate tests for alkaloids on 90 urine samples. For quinine and methadone, duplicate test results agreed (both test results positive or both negative) in only 84.4 % of the samples analyzed. With codeine and morphine greater agreement was obtained, but these data should be interpreted with caution because so few of the samples contained codeine or morphine.

TABLE II

RESULTS OF DUPLICATE TESTS FOR ALKALOIDS ON 90 SAMPLES

Narcotic drug	*% correlation*
Quinine	84.4
Methadone	84.4
Codeine	100
Morphine	97.8

If, however, large amounts of say methadone or other drugs were added to the urine, a positive test was always obtained, showing that the efficiency of the resin paper was much less important at higher drug levels. MULE[7] also has observed a fair degree of variability of alkaloid test results with the DOLE method. Still others[8] have reported variability in methadone test results.

Codeine masking. Identification and confirmation of alkaloids such as methadone, quinine and morphine is not too difficult because of the characteristic colors produced with spray reagents and/or the effect of time on the intensity of the colored spots. With quinine, for example, a distinct uniqueness (fluorescent spot) is obtained by spraying with sulfuric acid and observing the plate under UV light. With morphine, a blue-green spot was obtained after spraying with iodoplatinate. The intensity of the spot increased with time. After spraying with the silver nitrate reagent, the morphine spot disappeared and reappeared with heating to give a black color. Methadone gave a distinct color against a pink background. The intensity of the spot decreased with time.

Codeine, on the other hand, produced a purple spot upon spraying with iodoplatinate. The intensity of the spot did not vary with time (less than 30 min). This was unfortunate because many unidentified spots with about the same R_F value as codeine gave a purple time-independent spot following iodoplatinate spray. In certain instances it was actually impossible to identify codeine, because the codeine was

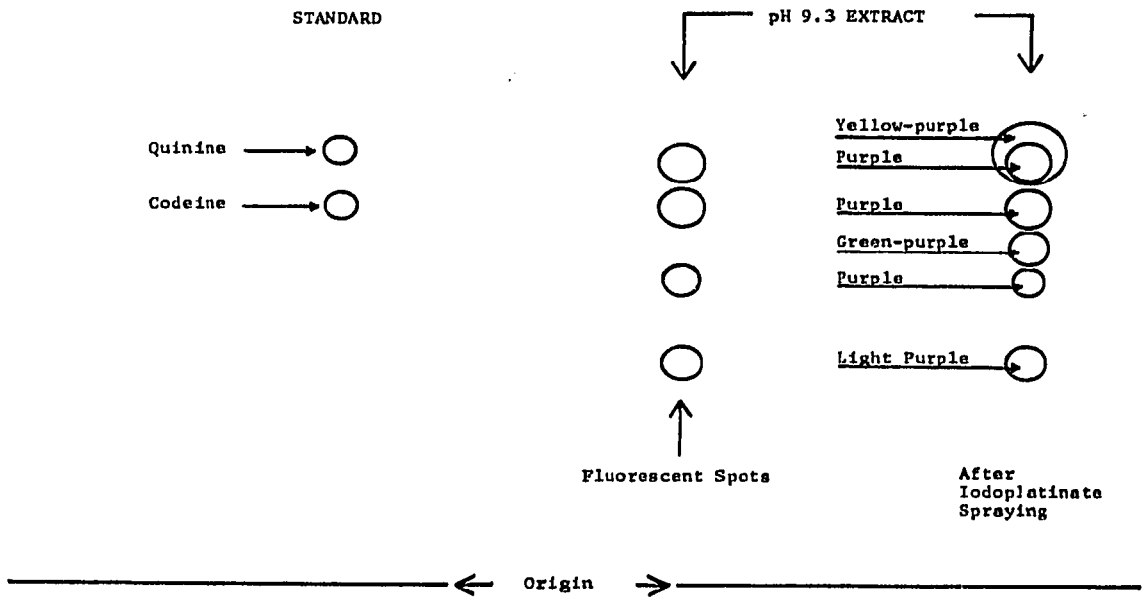

Fig. 1. Codeine masking by quinine metabolites.

masked by quinine metabolites. Fig. 1 demonstrates this behavior. The middle column shows the fluorescent spots obtained when large amounts of quinine were present in the patient's urine. One quinine spot matched that of the standard whereas the other three spots are quinine metabolites. One of these metabolite spots has the same R_F value as that of the codeine standard. After spraying with iodoplatinate, two additional spots appeared; all of the spots were various shades of purple. The spot which overlapped the codeine standard gave the same purple color as obtained with pure codeine.

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