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A RAPID AND COMPREHENSIVE SYSTEM FOR THE ROUTINE IDENTIFICATION OF DRUGS IN BIOLOGICAL MATERIAL BASED ON MICRO-PHASE EXTRACTION AND DRUG COLOUR PROFILES

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SUMMARY

The separation of basic, acidic and neutral drugs from propanol-2 extracts of serum, urine and tissue homogenates at different pH values using a micro-phase extraction technique is described. Following preliminary screening, the various drug-containing fractions obtained are further examined by two-dimensional thin-layer chromatography. The drugs present are identified with reference to documented standards with the aid of a drug colour profile system and R_F values relative to three different reference standards. By means of gas chromatographic analysis of the same extracts, semi-quantitative estimates of the amounts of drugs present, which are sufficiently accurate for clinical emergency purposes, can be made in many instances. The main advantages of the system are "clean" extracts with a minimum of background interference, rapidity (4–6 h for a complete analysis) and systematically documented and visually presented behaviour of drugs after spraying with various chromogenic and fluorogenic reagents, allowing the systematic identification of unknown substances.

INTRODUCTION

A general system for the qualitative and semi-quantitative analysis of all drugs that are extractable from biological fluids with organic solvents is described. The original procedure of Ramsey and Campbell[1] using small volumes of chloroform (50–100 μl) for the extraction of drugs from aqueous solutions has been modified in order to separate different classes of drugs by micro-phase chloroform extraction from aqueous solution at various pH values and, conversely, to separate drugs from organic solvents by using "micro-phases" that consist of aqueous buffers with various pH values. Combinations of these principles can be used in order to separate organic compounds from biological fluids.

The elimination of time-consuming concentration steps[1] and the fact that

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micro-phase extracts yield much "cleaner" chromatograms are among the main advantages of this method of extraction. A possible disadvantage is that not all drugs may be sufficiently soluble in the organic solvent used (chloroform–propanol-2) to ensure effective extraction, and in order to overcome this problem we have included a final exhaustive extraction with ethyl acetate. Owing to the use of preliminary toluene extraction of the acidified extract, the final ethyl acetate extract (B_2 fraction) also yields a relatively "clean" chromatogram with little background interference. The appearance of a drug in a particular fraction (or the drug distribution in more than one fraction) is used as a iditional evidence for establishing the identity of the drug.

The various fractions obtained in this manner (see Fig. 1) are next screened, after one-dimensional thin-layer chromatographic (TLC) separation[2], for the presence of drugs by a system of chromogenic sprays and only drug-containing fractions are

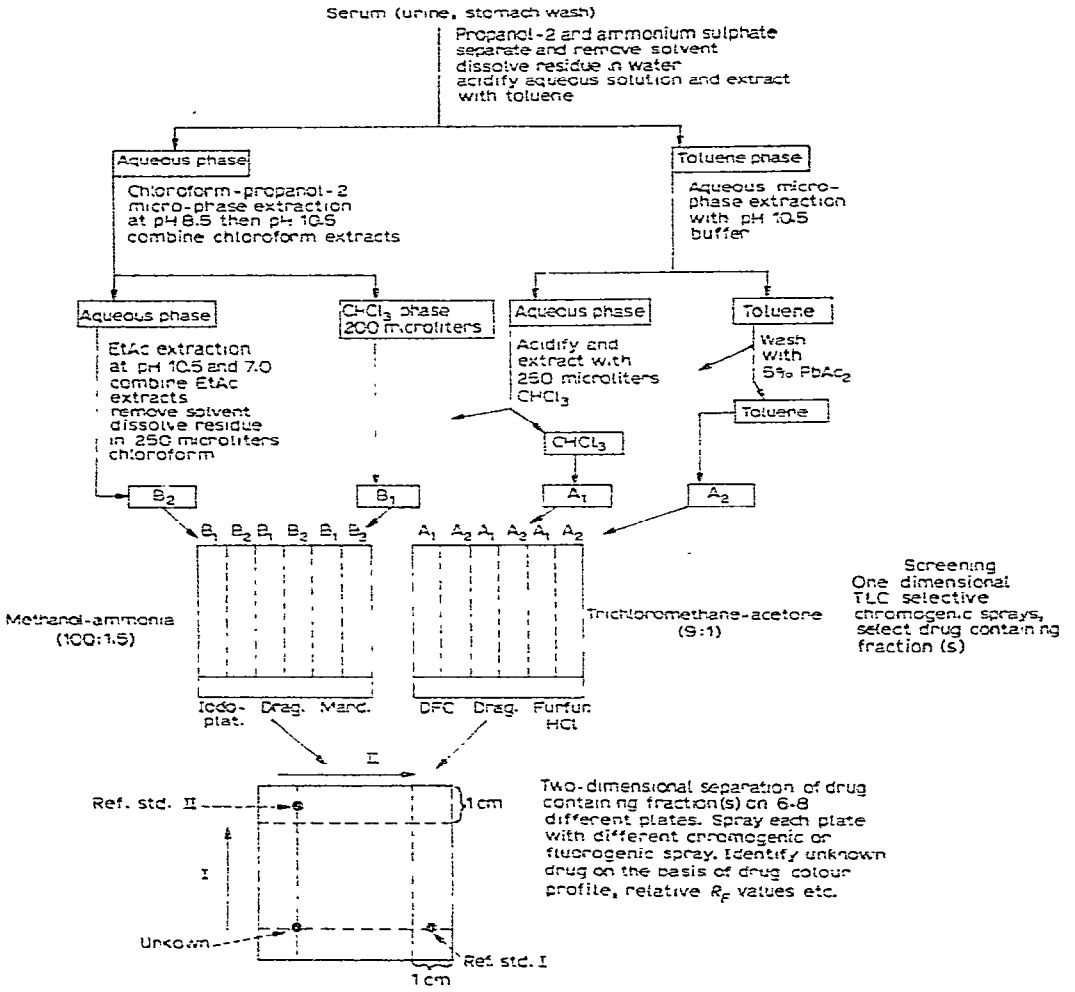

Fig. 1. Scheme for extraction of drugs.

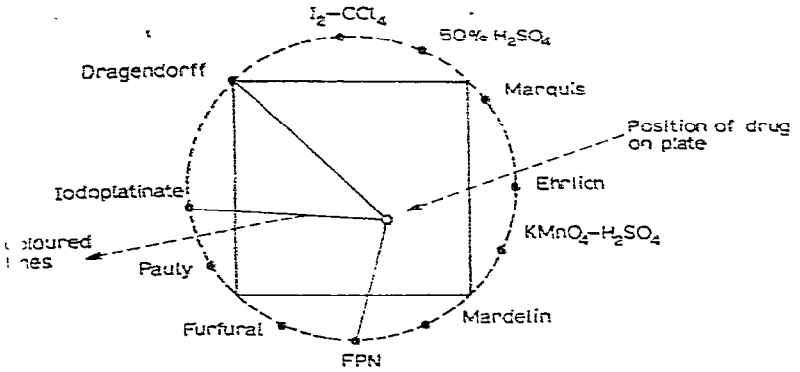

Fig. 2. Diagram for drug colour profile entry.

further investigated by means of two-dimensional TLC. In the final analysis, the two-dimensional TLC plates (generally 6–8 plates, sufficient extract being available to spot 10 plates) are each sprayed with a different chromogenic spray. The results are summarized and presented diagrammatically for purposes of comparison with pre-determined standards by entering the results of all sprays on a diagram representing the TLC plate with a circle drawn through the four corners of the plate (Fig. 2). The different sprays are indicated on the circumference of the circle and lines (corresponding to the colour obtained with the particular spray) are drawn so as to connect the position of the "spray" on the circle with that of the drug spot on the plate. In this manner, a "drug colour profile" is obtained in the form of various coloured lines radiating from the position of the drug spot on the plate, which further serves to identify and distinguish the particular drug from others. Also, a single visual presentation and record of the distinguishing properties of a particular drug are obtained for comparison with pre-determined standards. Identification is further facilitated by dividing the chromatogram into sections as previously suggested by Hundt[3] and by listing R_F values in each direction relative to two internal standards, as explained below.

The successful application of the method in practice depends on the availability of a well documented and readily accessible record system. In our laboratory, records of individual drugs that have been detected in urine and blood samples using this method have been compiled and these list the following details (entered on specially prepared forms): (a) nature of sample; (b) amount detectable; (c) fraction(s) in which drug appeared (see Fig. 1); (d) replica of the one-dimensional TLC plates obtained during the screening stage; (e) colour profile (see Fig. 2); (f) relative R_F values; (g) section in which drug appears; and (h) position of metabolites on plates and other information regarding metabolites.

The analysis of blood and urine samples may be complicated by the presence of one or several metabolites (often with similar chromogenic properties) and the situation may be further aggravated when an unfavourable concentration ratio of the parent drug to its metabolites exists, which often occurs when samples are collected long after ingestion of the drug concerned. However, as metabolites are often more polar than the parent compound and therefore tend to be more water soluble and less lipid soluble, they may not be concentrated in a particular fraction to an

extent similar to that of the parent compound and in this respect the present method offers distinct advantages. For example, if the parent compound is a basic, lipophilic drug, it can be expected to appear in the B_1 fraction. The more water-soluble and less lipid-soluble metabolites will then tend to be carried through mainly to the B_2 fraction. Despite the advantages offered by the method described, the identification of minute amounts of an unknown drug in a biological sample still constitutes a major challenge.

EXPERIMENTAL AND RESULTS

Reagents

Developing solvents (proportions by volume). Direction 1, ethyl acetate–methanol–ammonia (85:10:5); direction 2, *n*-butanol–acetic acid–water (70:20:10); screening, chloroform–acetone (9:1) and methanol–ammonia (100:1.5).

Reference standards. Direction 1, 10 mg each of methaqualone and pericyazine and 25 mg of salicylic acid per 10 ml of alcohol; direction 2, 10 mg each of methaqualone, pericyazine and metoclopramide per 10 ml of alcohol.

Chromogenic sprays. Mercury(II) chloride–diphenylcarbazone[4], FPN[4], potassium permanganate–sulphuric acid[5], Dragendorff[5], iron(III) chloride[5], Ehrlich[6], Pauly[5] and furfural[4] sprays were prepared as previously described.

Iodoplatinate spray was prepared by dissolving 0.25 g of hexachloroplatinic acid and 5 g of potassium iodide in 100 ml of 2 *N* hydrochloric acid and making the volume up to 500 ml with 2 *N* hydrochloric acid.

Iodine–carbon tetrachloride reagent was prepared by dissolving 1 g of iodine in 100 ml of carbon tetrachloride.

Marquis reagent consisted of 1 ml of formaldehyde solution in 10 ml of conc. sulphuric acid. View under UV light after spraying; heat and note colour changes.

Mandelin's reagent consisted of 1 g of ammonium vanadate dissolved in 100 ml of conc. sulphuric acid. Allow the sediment to settle before use.

Separation of drugs

Serum. A 5-ml volume of propanol-2 was added to 5 ml of serum in a 20-ml glass-stoppered test-tube and 3 g of solid ammonium sulphate were added so as to saturate the mixture. The "salted-out" layer of propanol-2 was separated by centrifugation, a drop of acetic acid added and then the liquid was evaporated to dryness in a rotary evaporator at 50°. The residue was dissolved in 2.0 ml of water, 0.4 ml of 0.5 *N* sulphuric acid and 0.1 ml of ethanol were added and the acidified solution was extracted with 5 ml of toluene using a shaking machine. The solution was centrifuged if necessary in order to separate the toluene phase, which was then filtered into a glass-stoppered nipple-tube marked "toluene" and the volume of this phase recorded.

(i) *Treatment of the aqueous phase.* A 0.3-ml volume of 1.0 *N* sodium hydroxide solution, 1.0 ml of phosphate buffer solution (pH 8.5), 1.0 g of sodium hydrogen carbonate and 1.0 g of sodium chloride were added and the mixture was shaken until saturated with respect to the added salts. After brief centrifugation, the clear aqueous phase was transferred into a glass-stoppered nipple-tube (Fig. 3) and 150 μl of chloroform–propanol-2 (9:1) were added. The inverted nipple-tube was shaken on a vortex mixer for 3 min, care being taken to ensure adequate dispersion of the organic

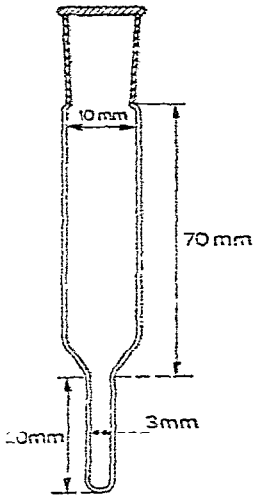

Fig. 3. Nipple-tube for micro-phase extraction of drugs.

phase. The mixture was then centrifuged at 700 *g* for 5 min until all of the chloroform phase had been transferred to the narrowed portion of the nipple-tube. As much of the supernatant liquid as possible was then transferred into a second nipple-tube without disturbing the chloroform layer in the first nipple-tube, which was marked "8.5". A 0.8-ml volume of 2.0 *N* sodium hydroxide solution was added to the aqueous phase in order to adjust the pH to above 10.5 and the micro-phase extraction procedure repeated with a further 150-μl portion of chloroform–propanol-2 (9:1), marking the organic phase "11.0" and retaining the aqueous phase.

The "8.5" and "11.0" fractions were combined so as to yield *ca.* 200 μl of chloroform phase, which was marked "B_1" and stored in a well-stoppered nipple-tube covered with a minute layer of water.

Finally, the aqueous phase was extracted twice with 5-ml portions of ethyl acetate–ethanol (20:1), once at the existing high pH value and once after adjusting the pH to 7.0 by the addition of 0.5 ml 2.0 *N* hydrochloric acid. The two ethyl acetate extracts were combined, dried briefly over a small amount of anhydrous sodium sulphate, filtered into a 50-ml round-bottomed flask and evaporated nearly to dryness on a rotary evaporator at 50°. The residue was dissolved in 230 μl of chloroform and marked "B_2". The solution was conveniently stored in a nipple-tube under a minute layer of water to prevent evaporation.

(ii) *Treatment of the toluene phase.* Exactly one fifth of the toluene phase was removed and stored separately for possible quantitative GC analysis later. The remainder of the toluene phase was further worked up by adding 0.2 ml of ethanol, filtering and extracting with 200 μl of 0.2 *M* borate buffer (pH 10.5) as above on the vortex mixer (3 min) and centrifuging in a nipple-tube. The toluene phase was retained, the aqueous phase (200 μl) transferred into a clean nipple tube and 50 μl of 0.5 *N* sulphuric acid were added in order to acidify the extract, which was then extracted once with 250 μl of chloroform–propanol-2 (9:1) by slowly expelling the air from a Pasteur pipette with the tip immersed in the aqueous phase so as to ensure mixing. Most of the top aqueous layer was removed with a Pasteur pipette, leaving

only a small aqueous layer to cover the chloroform phase collected in the lower, restricted portion of the nipple-tube, which was marked "A_1".

The remaining toluene phase was washed once with 3 ml of 5% lead acetate solution and the organic phase evaporated to dryness. The residue was dissolved in 250 μl of chloroform and marked "A_2". As before, this fraction was stored in a well-stoppered nipple-tube under a minute layer of water.

Urine. A 10-ml volume of urine was extracted with 10 ml of propanol-2 and 6 g of ammonium sulphate and the extract treated further as above so as to yield fractions B_1, B_2, A_1 and A_2 as before.

Stomach washings. A 20-ml volume of stomach wash fluid (preferably the first washing) was diluted with an equal volume of water, treated with sodium hydrogen carbonate until effervescence ceased and then centrifuged. A 5-ml portion of the clear supernatant liquid was then extracted as described for the extraction of serum.

Subdivision of fractions A_1, A_2, B_1 and B_2 for screening, confirmatory two-dimensional TLC identification and semi-quantitative GC analysis

At this stage, 200-μl portions (chloroform) of each of the above four fractions were obtained. For further analysis, each fraction was subdivided as follows (using suitable capillary tubes as receptacles: for screening, 50 μl: for two-dimensional TLC confirmation, 100 μl; and for possible semi-quantitative GC, 50 μl.

Preliminary screening

Two 10 × 5 cm thin-layer plates (Fertigplatten F254, Merck, Darmstadt, G.F.R.) were each divided into six channels by drawing lines with a spatula (Fig. 1). Fifteen microlitres of the acidic A fractions (three applications of A_1 and three applications of A_2) were applied to the channels on the first plate, as shown in Fig. 1. The basic B fractions were applied to the second plate in a similar fraction. The first plate (acidic fractions) was developed with chloroform–acetone (9:1) solvent and the second plate (basic fractions) using methanol–ammonia (100:1.5)[2]. Both plates were developed until the solvent front had reached a position at or near the end of the plate. After drying in an oven at 105° for 5 min, the three pairs of channels on each plate were sprayed separately with different chromogenic sprays as indicated in Fig. 1 (ref. 2). Suitable glass plates were used to cover the channels that were not being sprayed. Before spraying, the plates were viewed under UV light (254 and 360 nm) and any spots that did not appear on the documented serum (or urine) blank controls were carefully marked. The three pairs of channels on the acidic plate were then sprayed successively with mercury(II) chloride–diphenylcarbazone reagent[2] (for barbiturates and diphenylhydantoin), Dragendorff and furfural–hydrochloric acid sprays. The last reagent revealed meprobamate and other carbamates, while the Dragendorff reagent was included because of the wide range of drugs that react with it. The three pairs of channels on the basic plate were sprayed successively in a similar manner with iodoplatinate, Dragendorff and Mandelin reagents. Those channels which revealed no drugs after spraying as above were over-sprayed with potassium permanganate–sulphuric acid, iodine–carbon tetrachloride and 50% sulphuric acid on both plates so as to ensure that no drugs would be missed.

In this manner, the pattern of drugs and drug metabolites present is quickly recognized and, after some experience, the presence of certain commonly occurring

drugs (and, of course, barbiturates) can be discerned at this early stage and much time saved. Rapid confirmatory tests can then be carried out if necessary. However, in general it will be necessary or desirable to proceed to investigate the drug-containing fractions further by means of two-dimensional TLC, and for this purpose fractions that obviously contain the same drug were combined. In this manner, the actual number of fractions that were further investigated was usually limited to one or two. Although basic drugs would generally not be expected to appear in the acidic fractions A_1 and A_2, this does in fact happen owing to the slight solubility of the drug salts in organic solvents.

Final two-dimensional TLC separations and identification of drugs

Silica gel plates (5 × 5 cm Fertigplatten, Merck) were used. Light pencil lines were drawn 1 cm from the bottom and top and also from the left- and right-hand sides of the plate as in the method of Hundt[3] (Fig. 1). Reference standard solutions (1 μl of each) and the fraction to be investigated (20 μl) were applied to each plate as indicated in Fig. 1. The actual number of plates used and the amount of unknown solution applied to each depend on preliminary observations made during the screening stage and whether the fraction under investigation was obtained through a combination of two or more fractions, but in general six to eight plates were used per fraction examined and 10–20-μl volumes were applied to each plate. By using a suitable glass frame and clips that facilitated the chromatographic development process, all plates were developed in the first direction (8 min), dried in hot air, developed in the second direction (20 min) and finally dried in hot air.

One plate was then viewed under UV light (254 and 360 nm) and the positions of drug (and metabolite) spots marked with reference to standard documented controls obtained with drug-free samples in order to distinguish between drug spots and serum background spots, which, in the present system, gave very little interference. Thereafter, one plate each was sprayed with the following chromogenic sprays, omitting those that were observed to give negative results during the screening stage: iodoplatinate; Dragendorff; 50% sulphuric acid; Marquis; Ehrlich; potassium permanganate–sulphuric acid; Mandelin; FPN; furfural; and Pauly reagents.

In our laboratory, the general policy is to spray one plate each with iodoplatinate, Dragendorff, iodine–carbon tetrachloride and Mandelin reagents (four plates). The remaining plates are then sprayed with further chromogenic sprays according to indications obtained during the screening stage. It is useful to view the plates under UV light after spraying, especially with plates sprayed with Mandelin and Marquis reagents. With some sprays (Ehrlich and Marquis), the plates were heated for 10 min at 100° in an oven before inspection. If one or more drugs of the barbiturate group were present, the portion of the toluene extract that was retained for this purpose was analyzed immediately by GC[7].

Identification procedure

This procedure is based on the comparison of the unknown drug with documented drug records utilizing the following seven parameters: (a) R_F values relative to pericyazine, in two directions; (b) movement of the unknown compound on a 0–10 scale relative to the two other reference standards in each direction; (c) position on the plate (defined by the plate section); (d) drug colour profile; (e) drug distribution

amongst the different fractions (screening stage); (f) appearance of characteristic metabolites; and (g) fluorescent behaviour after spraying with certain reagents.

(a) R_F values relative to pericyazine standard. These values are defined for both directions as:

$$\frac{\text{Distance moved by unknown}}{\text{Distance moved by pericyazine}} \cdot 100$$

(b) Movement relative to the two other reference standards. One slow-moving and one fast-moving reference standard (in addition to pericyazine) were included in the standards for each of the two directions of chromatographic development. The position of the unknown compound was calculated in both directions with reference to one slow-moving and one fast-moving standard on a 0–10 scale in which the position of the slow-moving component was arbitrarily taken as zero and that of the fast-moving component as 10.

(c) Position on the plate (plate section). The 3 × 3 cm plate area where drugs may appear was divided into 16 square blocks (sections) by drawing four equally spaced lines in each direction[3]. The allocation of the unknown drug to a particular section in practice was best made by covering the plate with a transparent sheet similarly divided into 16 sections. The allocation of all documented drugs to such sections is particularly useful for documentation purposes as drugs can then be documented according to the section in which they appear as well as alphabetically and/or according to relative R_F values.

(d) Drug colour profile. Using the sketch in Fig. 2, the position of the unknown drug spot is marked on the central square area that has the same dimensions (5 × 5 cm) as those of the chromatographic plates used. Coloured radial lines corresponding to the colours obtained with the various sprays are then drawn from this point to the points marked for each chromogenic spray on the circumference of the circle as indicated. A small violet circle around the unknown drug spot is used to designate UV absorption by the drug while a double circle is used to signify fluorescence under UV light. Similar circles drawn around the spray positions marked on the circumference of the circle designate UV absorption and fluorescence after spraying with the particular reagent. This procedure summarizes the chromogenic behaviour of the unknown drug in a single graphic presentation that can be easily and rapidly compared with the documented records.

(e) Drug distribution among the different fractions observed during the screening stage. As stated, not all basic drugs appear in the basic fractions and not all acidic drugs appear in the acidic fractions, while many drugs appear in more than one fraction. The latter property can be used as a further distinguishing feature in the identification procedure, as exemplified by methaqualone, which appears typically in the A_2 and B_1 fractions.

(f) Drug metabolites. The presence of metabolites may, in some instances serve as additional confirmation of the presence of the parent drug compound, typical examples of such drugs being the phenothiazines, methaqualone and propoxyphene.

On the other hand, the presence of more than one metabolite (especially when more than one drug is present and when the concentrations of the metabolites are relatively high) can be very confusing. In such instances, the first step is to identify

the parent compound(s) by careful comparison of all unknown spots with the records in respect of the different parameters listed. Due consideration should be given to the fact that metabolites are generally more polar than the parent compounds and the chromatographic behaviour (including separation during the screening stage) must be interpreted in the light of such expected behaviour.

Semi-quantitative evaluation

It must be appreciated that the isolation procedures and working conditions used in this scheme were designed to cover the qualitative identification of a wide range of drugs and that these conditions are therefore generally not ideal for the quantitative determination of a particular drug. However, if quantitative results are available on pre-determined standards analyzed with various drug concentrations, upper and lower concentration limits of the drug present in a particular instance can usually be estimated. Although such results may not satisfy the analytical chemist, they may be of great significance to the clinician and such quantitative estimates should therefore always be attempted.

In practice, the amount of drug present in an aliquot of the extract (preserved for subsequent GC separation in a sealed capillary tube) is determined and the total amount of drug present in the sample is then calculated with due regard to the drug distribution pattern in the different fractions. Occasionally, unchanged drug samples are analyzed by GC for this purpose, but generally it is preferred to prepare a suitable derivative (acetyl, silyl or methyl). The derivatization procedures generally used were those described by Barrett[9], and if confirmatory evidence for the presence of certain groups of drugs was sought, his GC conditions were used. However, as a general screening procedure and for the possible quantitative evaluation of drugs, slow temperature programming over a wide range was preferred (150–300° at 4°/min). A 1 m × 2 mm I.D. column of 1% SE-30 on Chromosorb 750 was used and both free drugs and derivatives were studied, the results being compared with those for suitable blanks obtained by analyzing drug-free serum and urine samples.

DISCUSSION

The many advantages associated with micro-phase extraction methods[1,8] in drug separation techniques have not been fully appreciated and used in the past. This method significantly diminishes background interference by normal blood and urine constituents on gas chromatograms and on TLC plates, thus facilitating the recognition of the presence of drug spots and peaks and their subsequent identification. The drug colour profile data, on the other hand, enable the observer rapidly to compare a visual presentation of the chromogenic characteristics of the unknown drug with those of known standards.

In the practical application of the scheme, it is important to appreciate that a relatively small number of drugs (perhaps 30–40, depending on the locality) will be involved in up to 90% of the cases presented to the analyst. Successful identification of these drugs at the screening stage is greatly facilitated by carefully charted data, including R_F values, gas chromatograms and drug colour profiles, gathered in the course of personal handling of each of these drugs in the concentration ranges in which they may be expected to be present in actual samples. In addition, experience

TABLE I

EXPERIMENTAL PARAMETERS FOR THE IDENTIFICATION OF UNKNOWN DRUGS
DFC = Diphenylcarbazone.

Compound	R_F		*Section***	R_F *values*****, *two-dimensional*		*Amount detected*§ (*μg*)	*Colour profile*§§				*Fraction*§§§	*UV*
	*A**	*B**		*I*	*II*		*Iodoplatinate*	*Dragendorff*	*Mandelin*	*DFC*		
Analgesics												
Aspirin	0.075	0.75	14	14	114	1	—	—	G P	—	A_1	(+)
Cocaine	—	0.66	42/43	141	61	5	Pu	Or	—	—	B_1 A_2	(+)
Salicylic acid	0.062	0.78	14	15	100	1	—	—	B	—	A_1	+
Propoxyphene	—	0.72	43	150	100	1	Ru	Or	—	—	B_1	+ +
Paracetamol	0.05	0.77	24/34	95	152	1	—	—	—	—	B_2	+ +
Pentazocine	—	0.66	43/44	145	141	1	Ru	Or	Pu	—	B_1	(+)
Dipipanone	—	0.58	44/43	160	105	1	Ru Br	Or	—	—	B_1	(+)
Antihistamines												
Diphenhydramine	—	0.56	32/33	125	93	3	Br	Or	R Br	—	B_1	(+)
Promethazine	0.66	0.82	43/44	130	100	1	Bl G	Or	P	—	B_1 A_2	+
Hypnotics and sedatives												
Amobarbital	0.30	—	NA	NA		15	NA	NA	NA	+	A_1	(+)
Butobarbital	0.27	—	NA	NA		15	NA	NA	NA	+	A_1	(+)
Phenobarbital	0.22	—	NA	NA		15	NA	NA	NA	+	A_1	(+)
Diphenylhydantoin	0.16	—	34	126	171	1	—	—	—	+	A_1	(+)
Gluthethimide	0.47	—	NA	NA		15	NA	NA	NA	+	A_1	(+)
Pentobarbital	0.30	—	NA	NA		15	NA	NA	NA	+	A_1	(+)
Secobarbital	0.33	—	NA	NA		15	NA	NA	NA	+	A_1	(+)
Methaqualone	0.52	—	44	140	122	1	Ru Br	Or	B	+	A_2 (B_1)	+
Narcotics												
Codeine		0.32	23	68	79	1	G	Or	—	—	B_1	+
Meperidine	—	0.53	33/43	120	96	5	Pu Br	Or	—	—	B_1	(+)

Stimulants												
Caffeine	0.11	0.65	33/34	101	100	1	—	—				+
Tranquillizers												
Amitryptyline	—	0.52	44/$^{33}_{43}$	132	100	5	R Br	Or	Pu	—	B_1	+
Librium	0.04	0.75	34/33	110	112	1	R Br	—	—	—	B_1	+
Valium	0.37	0.81	44	143	115	1	Ru	Or	—	—	A_2	+
Chlorpromazine	—	0.5	33	130	104	1	Bl G	Or	P	—	A_2	+
Imipramine	—	0.51	34	121	98	1	Pu	Or	B	—	B_1	+
Perphenazine	—	0.62	33/34	82	98	1	Bl G	Or	Pu	—	A_2	+
Promazine	—	0.41	43/33	118	99	1	Bl G	Or	P	—	A_2	+
Thioridazine	—	0.48	33	116	108	1	Ru	Or	Pu	—	A_2	+
Trimeprazine	—	0.58	43	165	99	1	Br	Or	Or P	—	A_2	+
Fluorazepam	—	0.62	32	127	85	1	Br	Or	—	—	B_1	+
Miscellaneous												
Atropine	—	0.18	13/23	36	92	1	Pu G	Or	—	—	B_1	(+)
Chloroquine	—	0.23	33/23	101	99	1	Bl G	Or	—	—	B_1	+
Pyrimethamine	—	0.73	33/34	114	102	1	Bl G	Or	—	—	B_1	+
Dicyclomine	—	0.78	43	182	105	1	Ru	Or	—	—	B_1	—
Fenfluramine	—	0.56	33/34 23/24	97	103	10	Br	Or	—	—	B_1	(+)

* Screening: A, chloroform–acetone (9:1); B, methanol–ammonia (100:1.5).

** See text. NA = Not applicable; barbiturates and some related compounds when detected at the screening stage are further characterized by means of GC only[9].

*** R_f values defined relative to pericyazine as:

$$R_f = \frac{\text{distance moved by drug}}{\text{distance moved by pericyazine}} \cdot 100$$

§ Not necessarily the minimum detectable amount.

§§ R = red; R Br = red brown; Ru = rusty brown; Bl = black; G = grey; Pu = purple; Or = orange; P = pink; B = blue; Br = brown (chocolate).

§§§ Main fraction in which drug appears in the analytical scheme.

gained in handling actual hospital cases should be carefully documented for future reference, together with the above data, including gas chromatograms, and the significance of possible metabolites in the analytical scheme should be carefully noted.

Results obtained in the analysis of a number of representative drugs are listed in Table I. Obviously, R_F values are subject to variation within certain limits and therefore also the section under which a particular drug is listed, especially in borderline cases. Such variations should be taken into account in the practical application of the system, as the colour profile and the fractional distribution of drugs may be influenced by the amount present. The information given in Fig. 1 is based on results obtained with drug concentrations in the ranges indicated. In general, the analytical behaviour of drugs in the present system is independent of the biological source (serum or urine) from which the drugs were taken and for this reason no distinction is drawn in this respect in Table I.

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