SOLVENT SYSTEMS FOR THE IDENTIFICATION OF NARCOTICS BY PAPER CHROMATOGRAPHY

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A recent survey on paper chromatographic methods[1] for the identification of narcotics revealed that most of the solvent systems in use could be classified into three groups: acid, alkaline and neutral solvents. All the solvents of the first and second group and most of the neutral ones have an aqueous stationary phase and an alcoholic, particularly butanolic, mobile phase. Some authors use formamide, another polar substance, as an impregnant in combination with less polar mobile phases such as benzene, chloroform, etc.[2-4]. Chromatographic data in two isobutanol-containing solvents indicate[5,6] a satisfactory distribution of narcotics of the morphine group, and out of the 92 narcotics, 56 in the sulfate system and 68 in the phosphate system (mostly synthetic narcotics) have R_F-values between 0.7 and 1.0.

It is the object of this paper to report the results of a study of six paper chromatographic systems which have been developed to facilitate the separation and to produce a better distribution of R_F-values. The work has been limited to those narcotics which have practical value and to a few naturally occurring alkaloids.

EXPERIMENTAL

Material

Thirty-four commercially available narcotics were studied. The compounds were used without further purification.

Apparatus

Ascending chromatography was used for Systems 1, 2 and 3 as described previously[5]. For Systems 4, 5 and 6 an apparatus for descending chromatography in 30 × 30 × 61 cm glass jars was used.

Paper

Whatman No. 3 MM paper was used throughout. For Systems 1, 2 and 3, 36 × 46 cm cylinders were used with a line of application 2.5 cm from the lower end which accommodated 17 spots per sheet. Systems 4, 5 and 6 employed 23 × 46 cm strips with a line of application 7 cm from the upper end which held 9 spots per sheet.

Solutions

The narcotic (50 mg) was dissolved in methanol 10 ml (cryptopine in chloroform, narcotine in chloroform/methanol, narceine in *N* NaOH). This solution (10 μl = 50 μg) was applied to the salted and dried paper in Systems 1, 2 and 3 and to the untreated paper in Systems 4, 5 and 6.

System 1. Isobutanol–glacial acetic acid–water (100:10:24) on paper impregnated, with KH_2PO_4 (0.5 *M*, pH 4.2) was used. Equilibration took place overnight (16 h), development was carried out in 8 h[5].

System 2. Isobutanol–glacial acetic acid–water (100:10:24) on paper impregnated with $(NH_4)_2SO_4$ (2%, pH 5.3) was used. The equilibration and development steps were the same as in System 1.

System 3. Butyl acetate–glacial acetic acid–water (35:10:3) on paper impregnated with KH_2PO_4 (0.5 *M*, pH 4.2) was used overnight. Equilibration and ascending chromatography were carried out until the solvent front reached 30 cm (4–5 h).

System 4. Propyl alcohol–water–diethylamine (1:8:1) was used as the mobile phase on paper impregnated with light paraffin (BP 1948) in hexane 4%. The spots were applied to the untreated paper which was then impregnated by a technique which avoids washing out of the applied material[7]. Descending chromatography was done after an equilibration time (30 min) until the solvent front reached 26 cm (4–5 h).

System 5. A mobile phase comprised of ammonium formate in water (10%) saturated with *sec.*-octanol was used on a stationary phase comprised of paper impregnated with *sec.*-octanol in acetone (20%). The techniques of application and impregnation are the same as those used in System 4. The equilibration time was 30 min. Descending chromatography to a solvent front of 28 cm (5 h) was carried out.

System 6. Light paraffin (BP 1948)–diethylamine (9:1) was used as a mobile phase on paper impregnated with 20% formamide in acetone. The techniques of application and impregnation are identical with those used in System 4. No equilibration time is needed. Descending chromatography was carried out overnight (16 h). The chromatograms were sprayed immediately after drying at 105° for 5 min.

Detection

Except for System 6, for detection of the narcotics, the chromatograms were dried at room temperature. The solvent front and spots detected under U.V. light of 3660 and 2537 Å were marked and then the chromatograms were sprayed with potassium iodoplatinate reagent[5].

RESULTS AND DISCUSSION

Solvent systems

Systems 1 and 2 have been described previously[5,8,9]. Mobile phases similar to System 3, were studied by THIES AND REUTHER for the separation of alkaloids and in particular papaverine and narcotine[10]. Impregnation with phosphate improves the compactness of the spots and the reproducibility of the R_F-values. This solvent has a good stability in contrast to the mobile phases of Systems 1 and 2. Chromatograms can be prepared

with a solvent several weeks old. Re-use of the solvent, after replenishing the amount taken up by any previous chromatogram, had no detrimental effect on the reproducibility of R_F-values. For toxicologic and forensic case work the equilibration period can be omitted to save time.

System 4 resembles one of WALDI's solvent systems[11]. He worked with paper impregnated with petroleum of unspecified concentration. Replacing the propyl alcohol in this system by other alcohols, such as methanol, ethanol, isopropanol, isobutanol or butanol, produced a trend to lower R_F-values and slightly inferior spots. WALDI's chromatographic method proved unsuitable for the synthetic narcotics since high R_F-values were obtained. The basis of his method is the use of specific sets of R_F-values to identify a given compound, which sets are produced by systematically changing the polarity of the mobile phase by combining chloroform, cyclohexane and diethylamine in definite proportions. The stationary phase in WALDI's system is paper treated with formamide.

STEINEGGER AND OCHSNER[12] used a solvent with an aqueous phase containing formamide and sodium formate on octanol-impregnated paper for the separation of lobelia alkaloids. REICHELT[7] reported an improvement of the shape of alkaloidal spots if ammonium formate was added to the formamide used for the paper impregnation. These systems did not give either the desired R_F-spread or a good reproducibility with synthetic narcotics. It was found that 1% aqueous solution of ammonium formate on paper impregnated with *sec.*-octanol gives well shaped spots and that the R_F-values can be lowered by increasing the formate concentration. A 10% solution was chosen as the mobile phase of System 5 as giving the best spread of R_F-values. If the chromatograms are dried at room temperature and then sprayed with the potassium iodoplatinate reagent, a good "spectrum-of-colors" is obtained with various narcotics. Chromatograms from System 5 dried at elevated temperature before spraying show increased durability of the stained spots, but a loss of sensitivity, while chromatograms dried at room temperature develop a white-grayish background after spraying.

System 6 is a modification of another WALDI system[11] in which petroleum was replaced by light paraffin. Chromatograms from System 6 should be sprayed immediately after the expulsion of the adherent diethylamine vapours to prevent "after-run" of the mobile phase, which may falsify the R_F-values.

TABLE I

System	*Dielectric constant of major component*	
	Stationary phase	*Mobile phase*
1	80	31.7
2	80	31.7
3	80	5.14
6	84	2.2–4.7
4	3.4	80
5	2.2–4.7	80

The solvents used in this study can be arranged in terms of the polarity of their stationary and mobile phases as shown in Table I[13]. The degree of polarity is indicated by the dielectric constant of the major component as given in column 2 and 3 of Table I. Systems 1, 2, 3 and 6 have strongly polar stationary phases in combination with mobile phases of decreasing polarity. On the other hand, Systems 4 and 5 belong to the "reversed phase" category which have a strongly-polar-mobile-phase in contrast to a weakly-polar-stationary-phase.

R_F-values

The R_F-values of 34 narcotics and related compounds in six paper chromatographic systems are given in Table II. The values are averages of 8–10 spots chromatographed on different sheets. The greatest deviation from the average value was $\pm$ 0.04 R_F units. Compounds have generally lower R_F-values in System 3 compared to Systems 1 and 2 due to the lower polarity of butyl acetate and the higher content of acid. In System 6, the change of solubility properties has a greater effect than the lowering of polarity of the mobile phase. The number of compounds with high or unchanged R_F-values is equal to that with the low values as compared to the classical Systems 1 and 2.

Both "reversed phase" systems lead to an improved distribution of the R_F-values of synthetic narcotics. Many of these can be separated from each other and from the morphine group. The latter show a satisfactory distribution in Systems 1, 2 and 3, and travel "en bloc" with high R_F-values in Systems 4 and 5 and with low R_F-values in System 6. Myrophine is an exception to this rule since due to its myristic acid radical, it behaves rather like a fatty acid. Although System 1 does not separate. any of the synthetic narcotics any better than System 2, some members of the morphine group (*e.g.* morphine/oxymorphone, codeine/oxycodone and hydrocodone/oxycodone) show better separations in System 1. Other examples of group separations among the synthetic narcotics are indicated for the amidones, 23 to 27 in Table II which have low R_F-values in Systems 4 and 5, and high R_F-values in System 6; and for the phenylpiperidines 18 to 21 in Table II which have intermediate R_F-values in Systems 4, 5 and 6.

When choosing a solvent to separate related compounds, one should strive to find an R_F difference of at least 0.05 (ref.[14]) or better 0.08 (ref.[4]). This rule cannot be taken too literally, since separations in the lower R_F range can be achieved with smaller differences[15,16]. One hundred and ten pairs of compounds in Systems 1 and 2 can be found in Table II with an R_F difference of less than 0.08. Adding System 3 reduces this number of inseparables to fifty-three. With all six systems only seven pairs remain. In practice, some compounds with R_F-values in the higher range have to be added to this list. A total of 12 pairs should be considered as difficult to separate. Of these, four pairs include pyrahexyl along with myrophine, phenadoxone, alpha-acetylmethadol, and dipipanone. Pyrahexyl is the only narcotic which contains no nitrogen. Consequently it will not stain with the potassium iodoplatinate reagent and, being a phenol, can be identified by an azo-spray[17,18].

The best systems and the degree of separability of the remaining eight pairs are indicated in Table III.

TABLE II

R_F VALUES OF NARCOTICS IN SIX SOLVENT SYSTEMS

No.	Name of narcotic	R_F in system No.					
		1	2	3	4	5	6
1	Oxymorphone	0.21	0.10	0.06	0.93	0.80	0.08
2	Morphine	0.34	0.13	0.05	0.92	0.78	0.01
3	Oxycodone	0.34	0.22	0.16	0.92	0.81	0.16
4	Hydrocodone	0.46	0.26	0.23	0.95	0.81	0.08
5	Codeine	0.49	0.24	0.12	0.95	0.79	0.10
6	Ethylmorphine	0.66	0.45	0.33	0.94	0.77	0.18
7	Diamorphine	0.73	0.50	0.52	0.91	0.75	0.06
8	Thebaine	0.85	0.67	0.41	0.95	0.61	0.23
9	Hydromorphone	0.87	0.64	0.10	0.93	0.77	0.03
10	Benzylmorphine	0.94	0.84	0.62	0.97	0.34	0.30
11	Myrophine	0.98	0.98	1.00	0.01	0.01	0.97
12	Cryptopine*	0.56	0.34	0.53	0.00	0.55	0.10 (0.00)
13	Narceine*	0.80	0.75	0.62	0.82	0.78	0.00
14	Papaverine*	0.88	0.78	0.64	0.98	0.02	0.04
15	Narcotine*	0.88	0.78	0.72	s	0.02	0.10L
16	Cocaine	0.83	0.67	0.56	0.60L	0.71	0.67
17	Pyrahexyl	1.00	1.00	1.00	0.01	0.00	1.00
18	Anileridine	0.76	0.40	0.54	0.56	0.35	0.40
19	Pethidine	0.91	0.77	0.63	0.95	0.60	0.78
20	Alphaprodine	0.91	0.81	0.77	0.51	0.62	0.77
21	Alphameprodine	0.94	0.90	0.84	0.85	0.33	0.84
22	Ethoheptazine*	0.92	0.82	0.66	0.87	0.70	0.80
23	Normethadone	0.97	0.95	0.90	0.11	0.30	0.87
24	*l*-Dipipanone	0.99	0.98	0.91	0.00	0.07	0.98
25	Phenadoxone	0.99	0.98	0.90	0.02	0.00	0.98
26	Methadone	1.00	0.96	0.88	0.15	0.27	0.94
27	*l*-Isomethadone	1.00	0.96	0.90	0.02	0.19	0.93
28	Alpha-acetylmethadol	1.00	1.00	0.96	0.06	0.05	0.97
29	Propoxyphene	0.97	0.94	0.93	0.22	0.11	0.94
30	Diethylthiambutene	0.90	0.89	0.90	0.08	0.10	1.00
31	Levomoramide	0.97	0.95	0.87	0.0–0.5s	0.02	0.6s
32	Levallorphan	0.95	0.92	0.66	0.88	0.60	0.58
33	*dl*-Methorphan	0.99	0.92	0.78	0.47L	0.36	0.91
34	Phenazocine	0.95	0.92	0.91	0.67	0.07	0.82

s = streaking; L = elongated spot; (0.00) = some material remains at start.

* Not narcotic under international law.

In conclusion it should be pointed out that it is not the intention to present a complete scheme of separation of narcotics based on R_F-values. Although such schemes have been suggested, for example for 70 alkaloids using six solvent systems and nine sprays[4], it appears unrealistic to try to do the same for narcotics. Furthermore each scheme of this nature which is designed for a limited number of compounds becomes obsolete as soon as a new member shows up. If, on the other hand, an unknown narcotic is chromatographed in six systems one obtains a chromatographic pattern, which, if it does not lead to complete identification, at least it considerably

reduces the number of possibilities. The result of the chromatographic analysis will complement those of other methods like microcrystal and color tests, or U.V. -and I.R.-spectroscopy, depending on the kind and amount of material available. The chromatographic patterns of new or rare narcotics can be added to the existing collection increasing its value.

TABLE III

SEPARABILITY OF RELATED PAIRS OF NARCOTICS

Pair	*Best system*	*Separability*
Myrophine Phenadoxone	3	Overlap: blue spot of M on top of violet spot of P
Narcotine Papaverine	3	Little overlap: other systems available[8,19–21]
Dipipanone Phenadoxone	5	Separable
Methadone Normethadone	4	Little overlap: in System 5: spot of M red-violet, spot of N violet*
Myrophine Alpha-acetylmethadol	5	Separable
Myrophine Dipipanone	5	Separable
Alpha-acetylmethadol Dipipanone	5	Overlap: brown-violet spot of D on top of red-violet spot of A
Alpha-acetylmethadol Phenadoxone	5	Separable

* See also differentiation of metabolites[22].

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SUMMARY

Six solvent systems, two of them of "reversed phase" type, are recommended for the paper chromatographic separation of narcotics. A chromatographic pattern obtained in these systems will facilitate the identification process.

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