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Note

Analysis of drugs encountered in fatal poisonings using high-performance liquid chromatography and fluorescence detection

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High-performance liquid chromatography (HPLC) is now widely used for the analysis of drugs both in proprietary preparations and in extracts from biological tissues. In the majority of these applications, detection has been achieved by UV absorbance.

Fluorescence can offer an extremely sensitive method of detection but few of the drugs found in fatal poisonings have a high fluorescence quantum yield. However, large quantities of these substances are often isolated from body organs and fluids, and in these circumstances highly sensitive techniques are not required. A fluorimeter, particularly when coupled to some type of chromatographic separation, can then be used as a selective detector. Selective detection can be particularly useful in forensic toxicology where the identity of the drugs to be isolated is not always known beforehand, and also, where several drugs may be present in the same sample.

The general principles of HPLC with fluorescence detection have been described by Johnson *et al.*[1] and Slavin *et al.*[2]. Specific applications have been described for LSD[3] and imipramine[4] based on their native fluorescence. Fluorescence derivatization methods have been reported for a wide range of drugs[5,6]. This paper describes the fluorescence and chromatographic properties of compounds found in over 97% of cases of fatal drug overdose examined in Forensic Science Laboratories in Great Britain. This survey is almost entirely restricted to the parent compounds; with a few notable exceptions, large quantities of drug metabolites are rarely recovered in acute poisonings.

MATERIALS AND METHODS

Chromatography was carried out with either a 15-cm or a 25-cm Spherisorb, 5-μm silica column and a mobile phase consisting of methanol–2 *N* ammonium hydroxide–1 *N* ammonium nitrate (90:7:3) or a 15-cm octadecylsilane (ODS) coated Spherisorb, 5-μm silica column and a mobile phase consisting of methanol–water (35:65). A Beckman-Altex 110A pump was used to deliver the eluent at a flow-rate of 1 ml min^{-1}. A Perkin-Elmer 1000 filter fluorimeter, equipped with a 20-μl capacity square-section flow cell, was connected in series with a Cecil Instruments CE 2012 variable-wavelength UV monitor.

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Solutions of drugs were freshly prepared in methanol at about 1 mg ml^{-1}; such solutions are routinely used in this laboratory for both thin-layer and gas–liquid chromatography. Samples were injected onto the column by means of a Rheodyne 7120 valve fitted with a 20-μl injection loop.

Fluorescence spectra were recorded by trapping the solute in the flow cell after switching off the pump; this procedure was found to stop the eluent flow in less than 5 sec. The emission slit on the fluorimeter was set to its minimum value.

All operations were carried out at room temperature.

RESULTS AND DISCUSSION

Table I shows the wavelength of maximum fluorescence (λ_f) and the retention volumes (R_v) of basic and neutral drugs using a 25-cm silica column. The correspond-

TABLE I

CHROMATOGRAPHIC AND FLUORESCENCE PROPERTIES OF BASIC AND NEUTRAL DRUGS

R_v = retention volume from point of injection for a 25-cm column; λ_{ex} = 280 nm unless otherwise stated. The following drugs showed no fluorescence under the conditions used: amitriptyline, amphetamine, carbamazepine, chlormethiazole, cinnarizine, clomipramine, cocaine, cyclizine, dextromoramide, dextropropoxyphene, diamorphine, diazepam, diphenhydramine, dipipanone, dothiepin, doxepin, flurazepam, haloperidol, levorphanol, lignocaine, maprotiline, meprobamate, methadone, nitrazepam, nortriptyline, orphenadrine, oxprenolol, pethidine, phenacetin, strychnine and sulphathiazole.

Compound	R_v *(ml)*	λ_f *(nm)*	λ_{ex} *(nm)*
Benzocaine	3.7	397	
Chlordiazepoxide	3.9	weak	
Chloroquine	15.9	405	
Chlorpromazine	5.3	458	320
Codeine	7.5	weak	
Desipramine	10.3	410	
Desmethyldiazepam	3.9	weak	
Dihydrocodeine	11.3	weak	
Fluphenazine	4.7	475	320
Imipramine	6.1	412	
Lorazepam	3.9	weak	
Medazepam	3.9	weak	
Methotrimeprazine	5.0	456	320
Mianserin	4.2	412	
Methaqualone	3.8	weak	
Morphine	7.5	weak	
Oxazepam	3.9	weak	
Perphenazine	4.0	458	320
Pholcodine	8.3	weak	
Procaine	4.3	396	
Prochlorperazine	5.5	458	320
Promazine	6.7	452	320
Promethazine	5.5	452	320
Propranolol	5.5	398	
Protriptyline	10.9	402	
Sulphanilamide	3.7	398	
Temazepam	3.9	weak	
Thioridazine	6.3	466	320
Trifluoperazine	4.8	476	320
Trimeprazine	4.8	458	320
Trimipramine	4.4	412	

TABLE II
FLUORESCENCE PROPERTIES OF ACIDIC DRUGS
The retention volume was less than 2.0 ml in all cases. The following drugs showed no fluorescence under the conditions used: oxy- and thiobarbiturates, paracetamol, phenylbutazone, phenytoin and primidone.

Compound	λ_f *(nm)*	λ_{ex} *(nm)*
Diflunisal	430	320
Frusemide	422	320
Glutethimide	weak	280
Phenazone	weak	280
Salicylamide	430	320
Salicylic acid	420	320
Warfarin	418	320

ing data for acidic drugs on a 15-cm ODS column are set out in Table II. Wavelength maxima are uncorrected for the spectral response of the photomultiplier tube.

Nearly half of the drugs studied showed a fluorescence emission strong enough to enable the spectrum to be recorded (Fig. 1). For a small group of compounds, notably certain benzodiazepines and morphine and its congeners, the fluorescence was very weak but nevertheless sufficient to allow some identification via the retention volume. Drugs which showed no fluorescence under the conditions used are listed in the descriptive texts to the tables.

The selectivity achieved by the presence or absence of fluorescence is illustrated by Fig. 2 which shows the separation of a mixture of tricyclic antidepressant drugs, and sequential detection by UV absorbance and fluorescence. A further degree of specificity can be achieved by suitable choice of excitation and emission wavelengths.

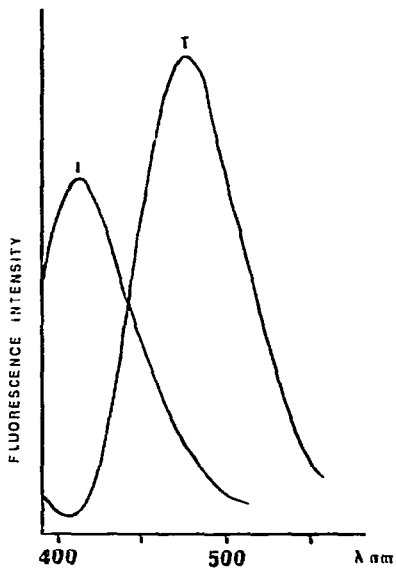

Fig. 1. Fluorescence emission spectra of trifluoperazine (T) (λ_{ex} = 320 nm) and imipramine (I) (λ_{ex} = 280 nm). (The lower wavelength cut-off at 390 nm is imposed by the limitations of the emission monochromator). Solvent: methanol–2 *N* ammonium hydroxide–1 *N* ammonium nitrate (90:7:3).

Thus the spectral resolution of a mixture can be achieved when incomplete chromatographic resolution occurs (Fig. 3).

The classification of drugs into fluorescent and non-fluorescent groups is partly dependent on the chromatographic and optical features of the equipment. For example, oxybarbiturates are fluorescent only in strongly alkaline solution (pH 12)[7], and it is doubtful if liquid chromatography could be successfully performed under such conditions. The non-fluorescence of other compounds can also be expected when no significant light absorption occurs at the excitation wavelength ($\lambda_{ex} \geqslant 280$ nm) (*e.g.*, amitriptyline, meprobamate).

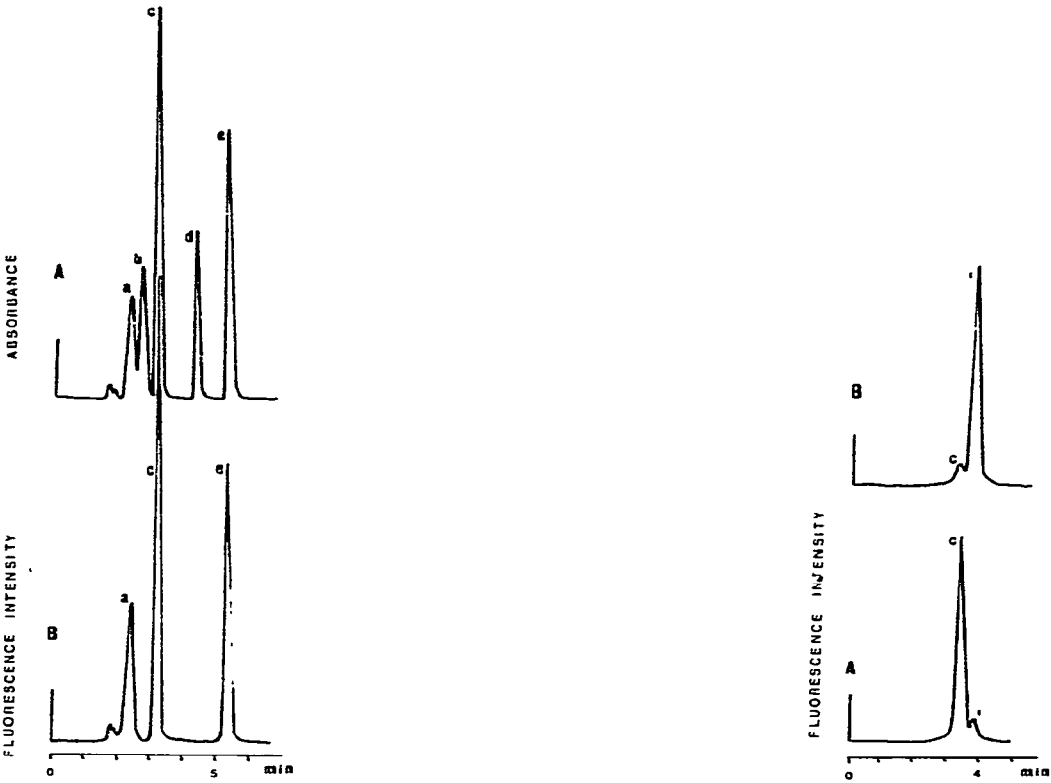

Fig. 2. The separation and sequential detection of five tricyclic anti-depressant drugs. A, UV absorbance, λ = 254 nm; B, fluorescence, λ_{ex} = 280 nm, λ_f = 410 nm. a = Trimipramine; b = amitriptyline; c = imipramine; d = nortriptyline; e = desipramine. Solvent: methanol–2 *N* ammonium hydroxide–1 *N* ammonium nitrate (90:7:3) (25-cm column).

Fig. 3. The spectral and chromatographic resolution of a mixture of chlorpromazine (C) and imipramine (I). A, λ_{ex} = 320 nm, λ_f = 460 nm; B, λ_{ex} = 280 nm, λ_f = 410 nm. Solvent: methanol–2 *N* ammonium hydroxide–1 *N* ammonium nitrate (90:7:3) (15-cm column).

The chromatographic separation of acidic drugs on ODS using the methanol–water system is very poor. Improved resolution can be obtained by using acidic solvents but most acidic drugs are not fluorescent under these conditions.

A majority of drugs do not have a sufficiently intense fluorescence for this to

offer the most sensitive means of detection. Notable exceptions are salicylic acid, salicylamide, protriptyline and chloroquine where the absolute limit of detection is in the nanogram range.

The work described here has been based on a filter fluorimeter. Considerably more discrimination could be achieved by using as a detector a fluorescence spectrometer equipped with an excitation monochromator. Fluorescent compounds could then be characterised by three parameters, namely the excitation spectrum, the emission spectrum and the retention volume.

REFERENCES

1 E. Johnson, A. Abu-Shumays and S. R. Abbott, *J. Chromatogr.*, 134 (1977) 107.
2 W. Slavin, A. T. Rhys-Williams and R. F. Adams, *J. Chromatogr.*, 134 (1977) 121.
3 B. B. Wheals, *J. Chromatogr.*, 122 (1976) 85.
4 P. A. Reece, R. Zacest and C. G. Barrow, *J. Chromatogr.*, 163 (1979) 310.
5 W. Dünges, *UV Spectrom. Group Bull.*, 5 (1977) 38.
6 R. W. Frei, W. Santi and M. Thomas, *J. Chromatogr.*, 116 (1976) 365.
7 L. A. Gifford, W. P. Hayes, L. A. King, J. N. Miller, D. T. Burns and J. W. Bridges, *Anal. Chem.*, 46 (1974) 94.