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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF BASIC DRUGS ON SILICA COLUMNS USING NON-AQUEOUS IONIC ELUENTS

II*. APPLICATION OF UV, FLUORESCENCE AND ELECTROCHEMICAL OXIDATION DETECTION

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SUMMARY

Unmodified silica columns together with non-aqueous ionic eluents give stable yet flexible systems for the analysis of basic drugs by high-performance liquid chromatography. Low-wavelength UV and fluorescence detection may be used, and fluorescence may be optimised by, for example, post-column pH change or derivatisation of some primary aliphatic amines with *o*-phthaldialdehyde. A novel feature is that electrochemical oxidation can be used for the detection of most analytes and this detection mode is thus discussed in detail. Retention and relative response data (UV, 254 nm and electrochemical, +1.2 V) have been generated for 462 compounds using a 125-mm Spherisorb S5W silica column and methanolic ammonium perchlorate (10 m*M*, pH 6.7) as eluent. This system can be used isocratically in qualitative analyses and also for quantitative work, when either the wavelength or the applied potential can be adjusted to optimise the response.

INTRODUCTION

Silica columns used with non-aqueous eluents such as methanol containing ionic modifiers provide stable yet flexible systems for the analysis of basic drugs by high-performance liquid chromatography (HPLC)[1], and the effect of alterations in eluent pH and ionic strength can be predicted from knowledge of the retention mech-

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anism[2]. Although UV absorption and fluorescence detection have been used widely in the analysis of basic drugs, oxidative mode electrochemical detection[3] has been restricted to compounds containing relatively easily oxidised groups such as phenolic hydroxyl or phenothiazine sulphur. However, the use of a glassy carbon working electrode in a wall-jet assembly together with a methanolic eluent containing oxidation-resistant ionic modifiers permits the extension of the technique to compounds such as secondary and tertiary aliphatic amines. The aim of the present paper is to discuss the application of silica column/non-aqueous ionic eluent systems to qualitative and quantitative analyses, with emphasis on the use of electrochemical detection.

EXPERIMENTAL

The reagents and experimental conditions were essentially as described previously[2]. Methanol (HPLC grade) was obtained from Rathburn (Walkerburn, U.K.) or from Fisons (Loughborough, U.K.), ammonium perchlorate from Aldrich (Gillingham, U.K.), perchloric acid (60%) from BDH, and *o*-phthaldialdehyde and 2-mercaptoethanol from Sigma (both Poole, U.K.). The nomenclature of the drugs studied follows that of Martindale[4].

Constant-flow reciprocating pumps were used with syringe-loading sample injection valves. Column effluents were monitored by UV absorption (Applied Chromatography Systems, Model 750/11, or Laboratory Data Control, Spectromonitor III), fluorescence (Kratos-Schoeffel, Model FS970, or Laboratory Data Control, Fluoromonitor III), or electrochemical oxidation using a V25 grade (carbonised at 2500°C) glassy carbon electrode (Le Carbone, Portslade, U.K.) in a wall-jet assembly. The construction of the cell and the electronics were similar to those described previously[5]. Saturated methanolic potassium chloride (analytical reagent grade) was used as the electrolyte in the reference electrode. Post-column reagent additions were performed at ambient temperature using a Kratos Model URS 050 post-column reaction system fitted with PTFE reaction coils (total volume 3.0 ml). Stainless-steel columns (125 or 250 × 4.9 mm I.D.) containing Spherisorb S5W silica (Phase Separations, Queensferry, U.K.) were obtained from Hichrom (Reading, U.K.) or packed from a methanol slurry and were used at ambient temperature at a flow-rate of 2.0 ml/min.

RESULTS AND DISCUSSION

Retention on silica column/non-aqueous ionic eluent systems is mediated primarily via cation exchange with surface silanols[2] and only positively charged species are retained. Clearly, this is useful since acidic/neutral compounds and non-protonated bases do not interfere. However, in addition to the sample preparation procedure, a further consideration is the detector used.

Modes of detection

At present, only UV absorption, fluorescence and electrochemical detectors offer the selectivity and sensitivity required in the analysis of drugs in body fluid extracts, and each shows flexibility in the ability to vary the absorption wavelength,

the excitation and emission wavelengths and the applied potential, respectively.

UV absorption and fluorescence detection. The use of UV absorption and fluorescence detectors is limited by the relative insensitivity of the former and the fact that few of the compounds of interest display natural fluorescence. One feature of non-aqueous ionic eluents is that UV absorption can be used down to *ca.* 205 nm thus giving enhanced sensitivity towards certain analytes, although the risk of interference is increased. Similarly, the fact that the eluent does not contain species that absorb UV light or quench fluorescence means that, with fluorescence detection, excitation wavelengths down to *ca.* 200 nm can be used and this gives enhanced sensitivity with certain compounds. Some analytes such as quinine and quinidine only display fluorescence at an appropriate pH, strongly acidic conditions being required to give a fluorescent species. However, poor peak shapes are obtained when using silica column/non-aqueous ionic eluent systems unless a basic eluent pH such as 8.3 is employed[2]. Since fluorescence monitoring is relatively insensitive to flow-rate changes, it is possible to alter the effluent pH post-column by simply using a strongly acidic "make-up" flow (Fig. 1). Note that additional information can be obtained if the analysis is repeated without "make-up" flow.

Post-column reagent addition may prove valuable in the detection of some primary aliphatic amines. Fluorescamine or 2-mercaptoethanol–*o*-phthaldialdehyde have been used to produce fluorescent products in the analysis of α-amino acids[6], although suffering the disadvantages of high cost and separate reagent addition with fluorescamine. The fluorescence detection of amphetamine using post-column derivatisation with 2-mercaptoethanol–*o*-phthaldialdehyde is illustrated in Fig. 2. Of the other compounds studied, phenylpropanolamine gave a response on this system while

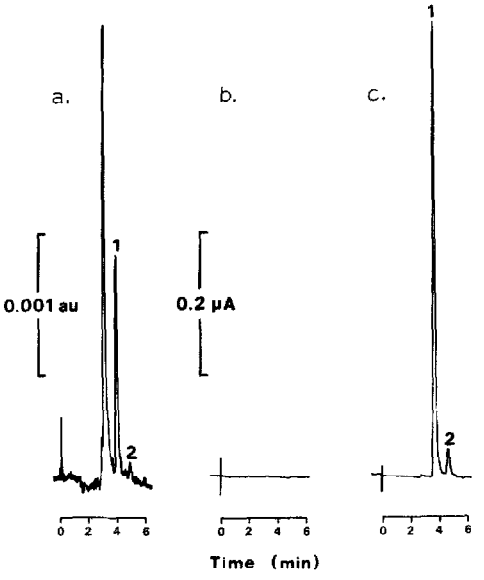

Fig. 1. Effect of effluent pH change on the fluorescence detection of quinine. Column, 125 mm Spherisorb S5W silica; eluent, ammonium perchlorate (20 m*M*) plus 60 ml/l methanolic sodium hydroxide (0.1 *M*), pH 8.3; injection, 20 μl of methanolic solution containing quinine (1) (0.2 mg/l) and dihydroquinine (2) (impurity). Detection: (a) UV, 230 nm; (b) fluorescence, excitation 250 nm, emission 470–700 nm; (c) as (b) but with 0.6 ml/min methanolic perchloric acid (60%) (1% v/v; *ca.* 0.1 *M*) added post-column.

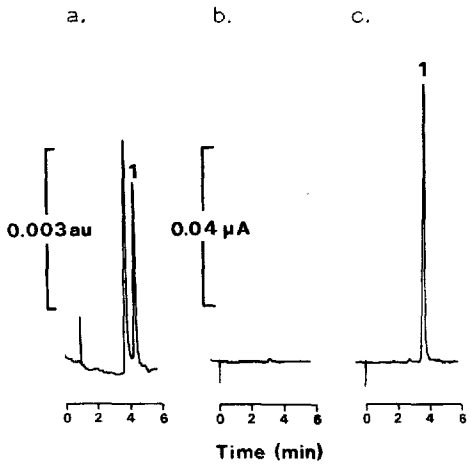

Fig. 2. Effect of post-column derivatisation with 2-mercaptoethanol–*o*-phthaldialdehyde on the fluorescence detection of amphetamine. Column, 125 mm Spherisorb S5W silica; eluent, methanolic perchloric acid (60%) (0.02% v/v; *ca.* 2 m*M*); injection, 100 μl of methanolic solution of amphetamine (1) (1 mg/l). Detection: (a) UV, 215 nm; (b) fluorescence, excitation 230 nm, detection 418–700 nm; (c) as (b) but with 0.6 ml/min *o*-phthaldialdehyde reagent added post-column. [The latter reagent was freshly prepared by diluting 1 ml of stock *o*-phthaldialdehyde solution (800 mg *o*-phthaldialdehyde plus 200 μl 2-mercaptoethanol in 10 ml methanol) to 100 ml with methanolic sodium hydroxide (0.2 *M*)].

tocainide and phentermine did not. The use of an ammonium perchlorate-modified eluent gave a higher background when used with the post-column derivatisation reagent than a perchloric acid-modified eluent, presumably due to the formation of fluorescent products by reaction with ammonia.

Electrochemical oxidation detection. The mechanisms of anodic oxidation of organic compounds are complex[7–9], although the first step is usually the removal of an electron to give a radical-cation. In the analysis of basic drugs the electron is invariably removed from a hetero-atom, commonly nitrogen, the potentials used being insufficient to oxidise the carbon skeleton of the molecule. The electrochemical response of a given compound can often be predicted from knowledge of the reactivity of its functional groups. In general, factors which lead to either increased availability of an electron or increased stability of the radical-cation lead to greater ease of oxidation. Thus:

(1) For aliphatic amines the ease of oxidation varies: tertiary > secondary > primary (Fig. 3a; C = amitriptyline, B = nortriptyline, A = amphetamine). This is attributable to the electron-donating properties of the alkyl groups and/or the stabilisation of the radical-cation by delocalisation of the charge. Tranylcypromine (Fig. 3b; D) is an aliphatic primary amine but is easily oxidised. Presumably the cyclopropyl group and/or the aromatic ring stabilise the radical-cation by charge delocalisation.

(2) Phenols, aromatic amines and heterocyclic aromatic compounds (Figs. 3b and 3c; E = methdilazine, F = imipramine, H = tyramine, I = tryptamine) are easy to oxidise and give good signals at 1.0 V applied or less. This ease of oxidation is probably due to resonance stabilisation of the radical-cation.

(3) Alicyclic tertiary amines (Fig. 3d; J = dipipanone, K = prolintane, L =

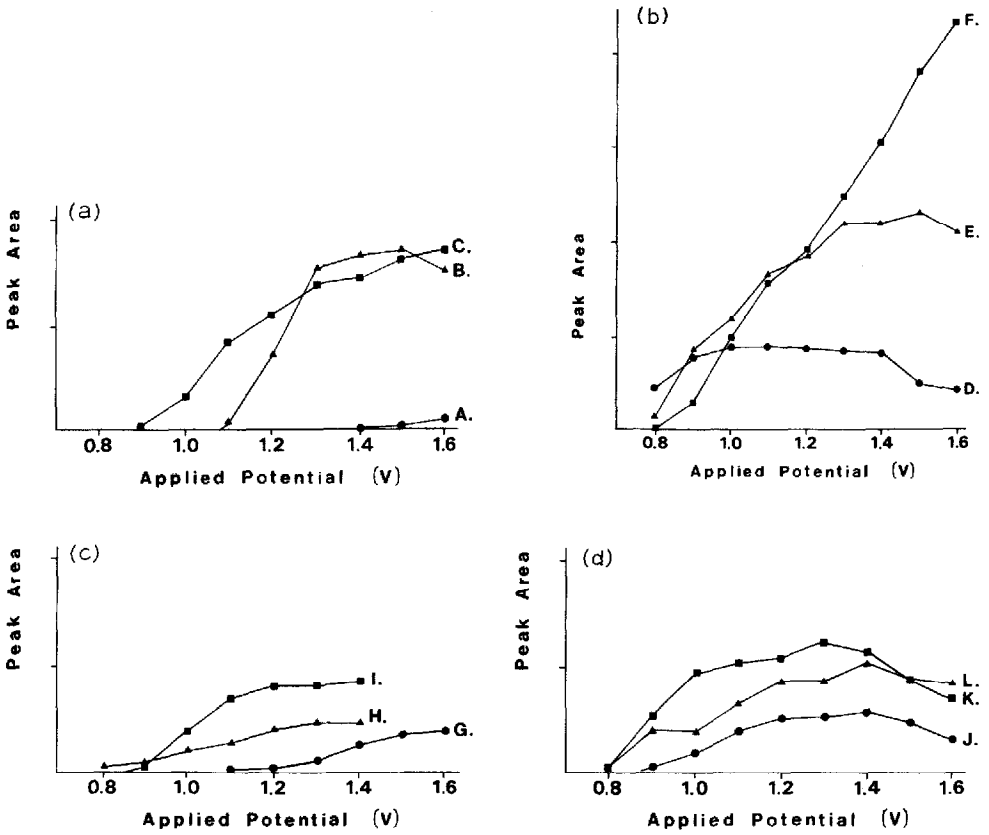

Fig. 3. Hydrodynamic voltammograms (signal *vs.* applied potential plots) for analytes containing different functional groups. Column, 125 mm Spherisorb S5W silica; eluent, methanolic ammonium perchlorate (10 m*M*) plus 1 ml/l methanolic sodium hydroxide (0.1 *M*), pH 6.7; injection, 20 μl of methanolic solutions containing each analyte (10 mg/l); detection, electrochemical oxidation at a range of applied potentials (results expressed as peak areas per 200 nmol injected).

Analyte		*Oxidisable group(s)*
A	Amphetamine	Primary aliphatic amine
B	Nortriptyline	Secondary aliphatic amine
C	Amitriptyline	Tertiary aliphatic amine
D	Tranylcypromine	Primary aliphatic amine adjacent to cyclopropyl group
E	Methdilazine	Phenothiazine sulphur; imidazoyl nitrogen; alicyclic tertiary amine
F	Imipramine	Imidazoyl nitrogen; tertiary aliphatic amine
G	Methylphenidate	Alicyclic secondary amine (six-membered ring)
H	Tyramine	Phenolic hydroxyl; primary aliphatic amine
I	Tryptamine	Indole nitrogen; primary aliphatic amine
J	Dipipanone	Alicyclic tertiary amine (six-membered ring)
K	Prolintane	Alicyclic tertiary amine (seven-membered ring)
L	Proheptazine	Alicyclic tertiary amine (five-membered ring)

proheptazine) are oxidised at a slightly lower applied potential than aliphatic tertiary amines. In addition, the five- and seven-membered rings are more reactive than the six-membered ring at 0.9 V applied.

(4) Alicyclic amines show a lower absolute response than the equivalent aliphatic amine (Figs. 3a, 3c and 3d). This may arise from the increased base strengths of the alicyclic compounds leading to a lower concentration of the oxidisable free base at the electrode.

(5) Some compounds (Fig. 3) show a reduced response at high applied potentials (1.5–1.6 V) possibly due to competition at the electrode with ammonia free base which is oxidisable at these potentials and present in large excess.

(6) Electrophilic substituents on an aromatic ring decrease the response obtained at a given potential when compared to that of an unsubstituted analogue. Thus, clomipramine and norclomipramine show a reduced response at 1.0 V applied in relation to imipramine (Fig. 4), although the effect for clomipramine is masked because the tertiary nitrogen is still oxidisable.

Although the use of a relatively high applied potential will maximise analyte response (Fig. 3), oxidation of the eluent and the electrode will be increased thus producing a higher background current. Baseline noise and drift are proportional to background current and thus higher applied potentials may decrease the signal-to-noise ratio. This is reflected in the detection limits of compounds with different functional groups (Table I). Note that ammonium perchlorate was used as the ionic modifier in this work since it has negligible UV absorption and the perchlorate ion is resistant to oxidation at the potentials used thus limiting the background current. Whether the use of alternative ionic modifiers would influence either the background current or the response of different oxidisable moieties is a question for further study.

Both the background current and response from basic moieties are dependent

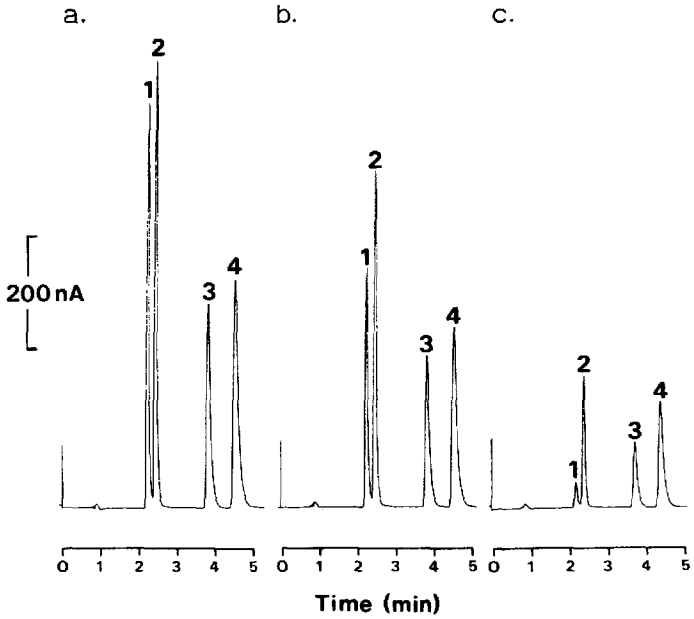

Fig. 4. Influence of electrophilic substitution on the electrochemical response of imipramine. Injection, 20 μl of methanolic solution containing norclomipramine (3-chlorodesipramine, 1), desipramine (2), clomipramine (3-chloroimipramine, 3) and imipramine (4) (all 10 mg/l). Detection: electrochemical oxidation, (a) +1.2 V, (b) +1.1 V, (c) +1.0 V. See legend to Fig. 3 for chromatographic conditions.

TABLE I

OXIDATION POTENTIALS AND LIMITS OF SENSITIVITY FOR CERTAIN FUNCTIONAL GROUPS

See legend to Fig. 3 for chromatographic conditions.

Functional group	*Optimum oxidation voltage (V)*	*Approximate detection limit (ng)*
Phenol, aromatic amine	0.7	0.1
Phenothiazine sulphur	0.8	0.1
Imidazoyl nitrogen, indole	0.9	0.2
Tertiary aliphatic amine	1.0	0.5
Secondary aliphatic amine	1.2	2
Primary aliphatic amine	1.6	20
Pyridyl nitrogen, quaternary ammonium compound, amide	>1.6	–

on pH. Aliphatic amines are only oxidisable when present in the non-protonated form, and an eluent pH of 6.7 is a compromise between retention, peak shape and response[2]. Increasing the eluent pH produces a higher absolute response for oxidisable amines since the non-protonated form is favoured, giving more oxidisable molecules at the electrode. However, the background current also increases due to oxidation of hydroxyl ions and ammonia free base. Enhanced selectivity and sensitivity can be obtained for compounds such as phenothiazines which have non-basic oxidisable moieties by using a low pH eluent. In practice, however, these compounds can be detected selectively at pH 6.7 using a lower applied potential (Fig. 5).

A number of materials have been used as the working electrode in oxidative-mode electrochemical detection, including platinum, glassy carbon, pyrolytic graphite and wax–graphite mixtures[3]. We have found that, for the chromatographic systems under discussion, glassy carbon[10] provides a suitable electrode material. However, the nature of the glassy carbon can influence the response of some compounds (Fig. 6). V10 and V25 grades of glassy carbon were obtained from the same source and the electrodes had been through identical polishing procedures before use. One difference between the materials was the temperature to which they were heated during production (1000°C for V10 and 2500°C for V25, information from Le Carbone). The background current from the V25 electrode was twice that from the V10 electrode. The V10 electrode showed a good response for phenols, aromatic amines and heterocyclic aromatic compounds but a poor response to aliphatic amines: 1.5 V had to be applied to obtain an amitriptyline response equivalent to that obtained on a V25 electrode at 1.0 V, whereas nortriptyline produced only a very small response even at 1.6 V.

A common problem in the use of electrochemical detection is variability in response attributed to electrode deactivation. Although deactivation was observed on the system described here, the process was slow and did not present a serious problem. Thus, the response characteristics of the electrode varied with time, the effect being particularly noticeable for nortriptyline for which 1.2 V applied is close to the oxidation threshold (Fig. 7). The initial decrease in response was rapid as the electrode stabilised, followed by a slow reduction over a period of weeks or months.

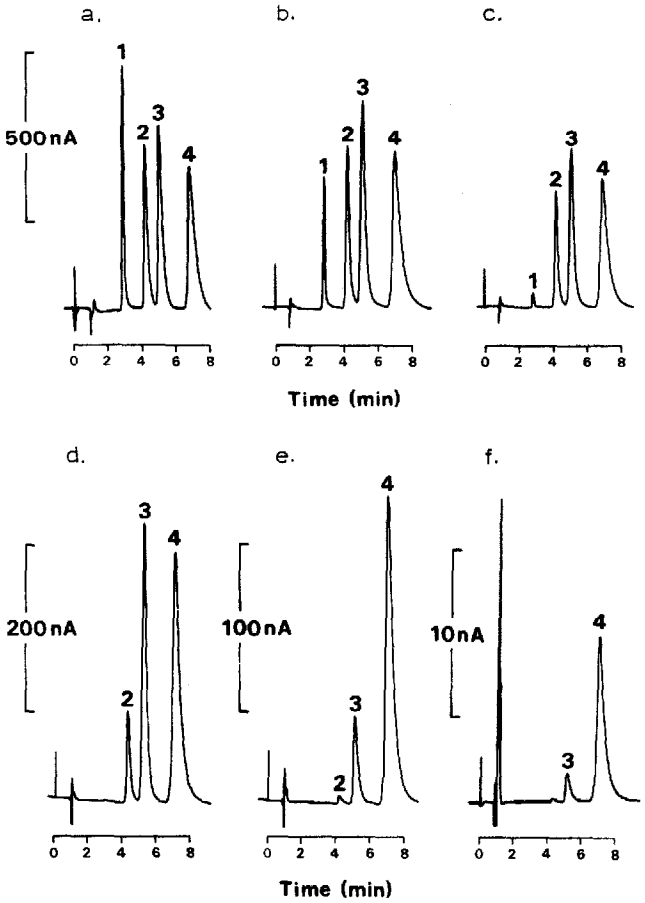

Fig. 5. Influence of applied potential on the electrochemical response of some test compounds. Injection, 20 μl of methanolic solution containing nortriptyline (1), amitriptyline (2), imipramine (3) and methdilazine (4) (all 10 mg/l). Detection: electrochemical oxidation, (a) +1.2 V, (b) +1.1 V, (c) +1.0 V, (d) +0.9 V, (e) +0.8 V, (f) +0.7 V. See legend to Fig. 3 for chromatographic conditions.

After the initial rapid change, the rate of reduction of response even for nortriptyline was not normally measurable over a working day. The response can be regenerated by:

(1) Applying a reverse potential to the working electrode. This may regenerate the original signal but there is a risk of corroding the stainless-steel auxiliary electrode.

(2) Removing the electrode and polishing it for *ca.* 2 min on a felt pad with an aqueous slurry of 1μm alumina. This returns the electrode to its original state and has proved the most satisfactory method of maintaining uniform performance. However, this process was usually only necessary after use for more than one month.

(3) Using the detector at 0.1 V higher applied potential than normal. Thus, 1.3 V applied on a partially deactivated electrode was almost equivalent to 1.2 V on a clean electrode (Fig. 8). Changing the applied potential is a convenient method of renewing the response when it is not practical to clean the electrode.

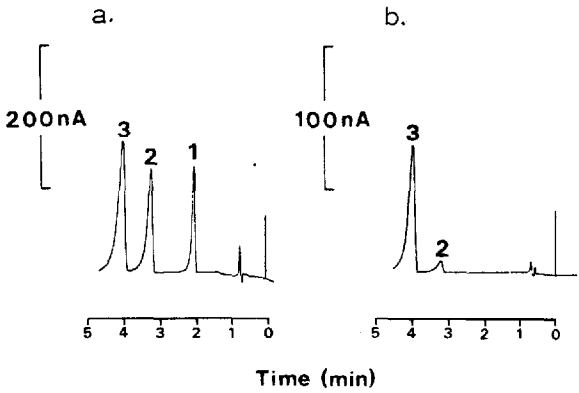

Fig. 6. Influence of the nature of the electrode on the electrochemical response of nortriptyline (1), amitriptyline (2) and imipramine (3). Injection, 20 μl of a methanolic solution containing each compound (10 mg/l). Detection: electrochemical oxidation, +1.2 V, (a) V25 electrode, (b) V10 electrode. See legend to Fig. 3 for chromatographic conditions.

We have not studied systematically the response obtained from commercially available detectors. However, the Metrohm Model 656 detector gave a similar response to that illustrated in Fig. 5 at 1.2 V but at an applied potential of 1.4 V. Secondly, this cell showed more rapid deactivation in routine use than that discussed

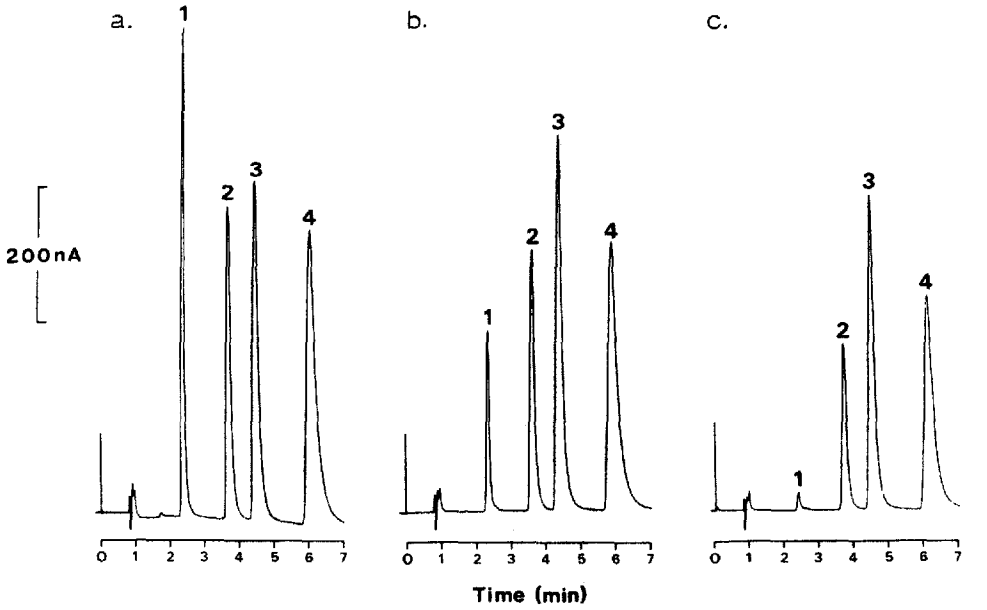

Fig. 7. Electrochemical electrode deactivation in routine use. Injection, 20 μl of methanolic solution containing nortriptyline (1), amitriptyline (2), imipramine (3) and methdilazine (4) (all 10 mg/l). Detection: electrochemical oxidation, +1.2 V. (a) Freshly polished electrode after 1 h equilibration, background current 1 μA; (b) after overnight equilibration, background current 280 nA; (c) after 3 months regular use for the analysis of body fluid extracts, background current 150 nA. See legend to Fig. 3 for chromatographic conditions.

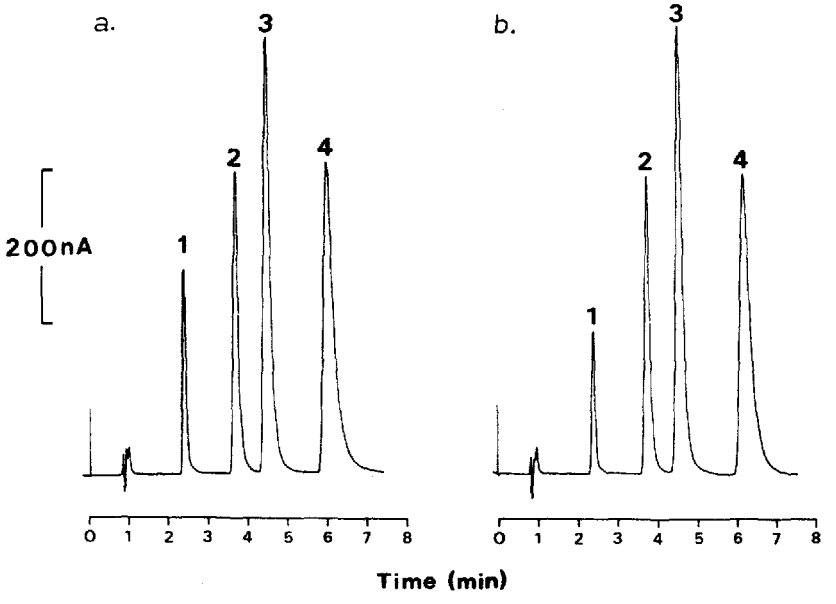

Fig. 8. Restoration of analyte response on a deactivated electrode by use of a higher applied potential. Injection, 20 μl of methanolic solution containing nortriptyline (1), amitriptyline (2), imipramine (3) and methdilazine (4) (all 10 mg/l). Detection: electrochemical oxidation, (a) + 1.2 V using a "clean" electrode, background current 280 nA, (b) + 1.3 V using a deactivated electrode [the same as in Fig. 7(c)], background current 250 nA. See legend to Fig. 3 for chromatographic conditions.

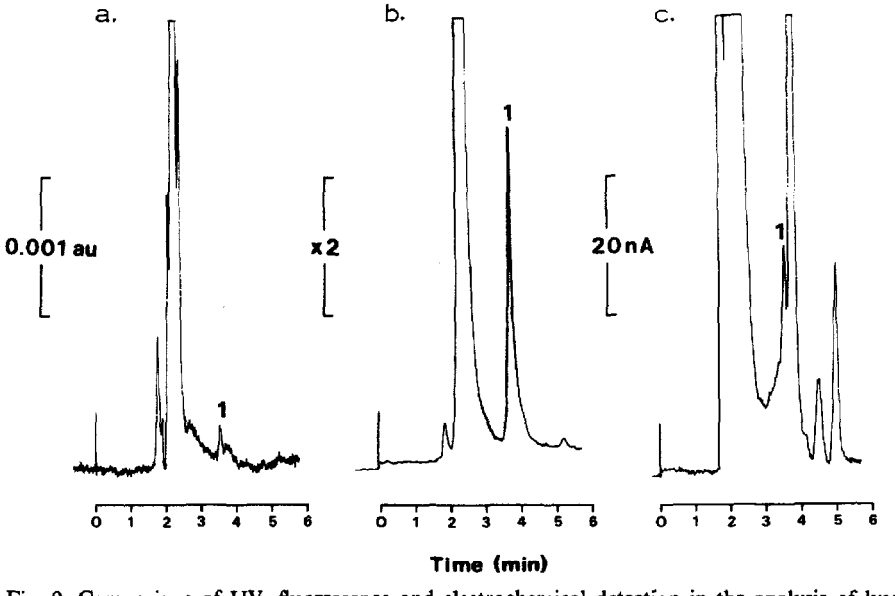

Fig. 9. Comparison of UV, fluorescence and electrochemical detection in the analysis of lysergic acid diethylamide (LSD, 1). Column, 250 mm Spherisorb S5W silica; eluent, methanolic ammonium perchlorate (10 m*M*) plus 1 ml/l methanolic sodium hydroxide (0.1 *M*); injection, 20 μl of concentrated extract of urine sample (1 ml) containing LSD (10 μg/l). Detection: (a) UV, 325 nm; (b) fluorescence, excitation 308 nm, emission 370–700 nm; (c) electrochemical oxidation, +0.8 V.

above, especially when analysing extracts of post-mortem specimens, although polishing the electrode using an alumina slurry again restored the response.

Comparison of detection methods. The measurement of LSD provides a good example of the additional selectivity and sensitivity which can be obtained using fluorescence even when compared to electrochemical detection (Fig. 9). Thus, UV absorption does not provide adequate sensitivity despite the relatively high extinction coefficient of LSD at 326 nm, and although electrochemical oxidation provides good sensitivity, selectivity is poor even at relatively low applied potentials. On the other hand, fluorescence detection gives good sensitivity and selectivity.

Application to qualitative and quantitative analyses

The chromatographic system described has been designed primarily for the measurement of basic drugs in body fluid extracts. The range of compounds that may be encountered is such that it is not possible to devise a single extraction method that is universally applicable. However, the fact that only positively charged species are retained means that extensive extract "clean-up" is not normally required. Selection of the chromatographic and detection conditions will depend on the individual analyte. When simple solvent extractions are employed with direct analysis of the resulting extract[11,12], the inclusion of a proportion (10–20% v/v) of iso-octane (2,2,4-trimethylpentane), diethyl ether or methyl *tert.*-butyl ether in the eluent may minimise lipid accumulation on the column. The use of a similar proportion of water in the eluent is advisable if aqueous solutions such as those resulting from the precipitation of plasma protein with methanol are to be analysed directly, although this will preclude the use of electrochemical detection for secondary aliphatic amines and possibly other compounds.

Although the number of analytes retained is maximised using a strongly acidic eluent this has disadvantages, notably the fact that compounds whose only oxidisable group is an aliphatic amine do not respond to the electrochemical detector when fully protonated[2]. As discussed previously, a methanolic ammonium perchlorate (10 m*M*, pH 6.7) eluent is a compromise between retention, peak shape and response, the use of an oxidation potential of 1.2 V on the V25 electrode ensuring that all retained analytes respond except those containing only a primary aliphatic amine, quaternary ammonium, N-oxide or other oxidation-resistant function. A convenient way of obtaining the working eluent is to prepare 1 l of methanolic ammonium perchlorate (0.1 *M*) + 10 ml/l methanolic sodium hydroxide (0.1 *M*) and to dilute this 1 in 10 with methanol before use. Independent measurement of the pH is not normally necessary.

The routine use of UV and electrochemical detectors in series has a number of practical advantages, notably the fact that the response ratio from the two detectors gives an additional identification parameter which is largely independent of retention time. The response to each detector originates from a different part of the molecule and thus unless co-eluting analytes have very similar chromophores and oxidisable group(s) they should be distinguishable on the basis of their response ratios. Retention and relative response data (UV, 254 nm and electrochemical, +1.2 V) have been generated for 462 compounds using a 125 mm silica column and methanolic ammonium perchlorate (10 m*M*, pH 6.7) as eluent (Tables II and III). Obviously it was not practicable to measure response ratios at all the other possible

TABLE II

RETENTION AND RESPONSE DATA (UV, 254 nm AND ELECTROCHEMICAL OXIDATION, +1.2 V) FOR THE COMPOUNDS STUDIED IN ALPHABETICAL ORDER OF COMPOUND

See legend to Fig. 3 for chromatographic conditions. Key: k' = column capacity factor; relative retention time = retention relative to imipramine; detector response ratio = peak height ratio electrochemical: UV (μA/a.u.) and coded as follows:

Ratio	*Code*
10 or less	A
11–20	B
21–50	C
51–100	D
110–200	E
210–500	F
510–1000	G
1100 or more	H

* = Tailing peak; – = no electrochemical response at +1.2 V; # = no UV absorption at 254 nm. Methadone Metabolite 1 is 2-ethyl-1,5-dimethyl-3,3-diphenyl-1-pyrrolidinium, and Methadone Metabolite 2 is 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline.

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
1-Phenylethylamine	0.44	1.2	–	–
10-Hydroxyamitriptyline	0.80	2.9	37	C
10-Hydroxyimipramine	0.90	3.4	41	C
10-Hydroxynortriptyline	0.58	1.8	15	B
11-Hydroxyclomipramine	0.76	2.9	50	C
2-Hydroxydesipramine	0.45	1.2	40	C
2-Hydroxyimipramine	0.83	3.1	67	D
2-Phenylethylamine	0.44	1.2	–	–
3-Methoxy-4,5-methylenedioxyamphetamine	0.41	1.1	–	–
3-Monoacetylmorphine*	0.82	3.1	400	F
4-Hydroxypropranolol	0.43	1.1	120	E
6-Monoacetylmorphine*	0.91	3.6	410	F
Acebutolol	0.48	1.4	6	A
Acepromazine	1.02	4.1	42	C
Acetanilide	0.22	0.1	–	–
Acetazolamide	0.22	0.1	–	–
Acetophenazine	0.58	1.9	43	C
Acetorphine	0.27	0.4	210	F
N-Acetylprocainamide (NAPA)	0.78	3.0	24	C
Adiphenine	0.53	1.8	950	G
Ajmaline*	0.72	2.8	110	E
Allylprodine	0.57	2.0	810	G
Alphacetylmethadol	0.58	1.7	440	F
Alphameprodine*	0.65	2.4	1100	H
Alphamethadol	0.62	2.1	540	G
Alphaprodine*	0.74	2.8	1300	H
Alprenolol	0.44	1.2	13	B
Alverine	0.57	1.8	930	G
Amethocaine	0.60	2.0	360	F

TABLE II (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Amidopyrine	0.27	0.3	79	D
Amiodarone	0.66	2.4	23	C
Amitriptyline	0.83	3.3	52	D
Amotriphene	0.60	2.0	14	B
Amphetamine	0.36	0.9	–	–
Anileridine	0.40	1.1	95	D
Antazoline	0.57	1.8	72	D
Apomorphine*	0.89	3.7	82	D
Atenolol	0.45	1.3	150	E
Atropine*	0.94	3.9	650	G
Azacyclonal	0.43	1.2	4	A
Bamethane	0.37	0.9	420	F
Benactyzine	0.52	1.7	640	G
Benperidol	0.41	1.1	97	D
Benzethidine	0.46	1.4	990	G
Benzhexol	0.55	1.8	410	F
Benzocaine	0.23	0.1	43	C
Benzoctamine	0.51	1.7	73	D
Benzoylecgonine*	0.37	0.9	32	C
Benzphetamine	0.43	1.2	600	G
Benzquinamide	0.26	0.3	260	F
Benztropine*	0.94	3.7	120	E
Benzylmorphine*	1.03	4.4	210	F
Betacetylmethadol	0.57	2.0	570	G
Betahistine	0.82	3.1	5	A
Betameprodine	0.53	1.8	800	G
Betamethadol	0.63	2.3	370	F
Betaprodine	0.67	2.6	940	G
Bezitramide	0.23	0.2	43	C
Bretylium*	1.09	4.3	–	–
Bromhexine	0.27	0.4	65	D
Bromodiphenhydramine	0.75	2.7	420	F
Bromperidol	0.46	1.3	46	C
Brompheniramine	0.98	4.1	75	D
Brompromazine	0.93	3.7	22	C
Brucine*	2.34	11.1	45	C
Buclizine	0.32	0.7	490	F
Bufotenine	0.78	3.1	180	E
Buphenine	0.37	0.9	410	F
Bupivacaine	0.36	0.9	670	G
Buprenorphine	0.28	0.4	430	F
Butacaine	0.45	1.2	120	E
Butaperazine	0.95	3.4	27	C
Butethamate	0.56	1.9	1600	H
Butriptyline	0.72	2.7	890	G
Caffeine	0.25	0.2	–	–
Carbinoxamine*	1.16	4.7	80	D
Carphenazine	0.57	1.7	25	C
Cephaline*	1.68	7.7	340	F
Chlorcyclizine	0.68	2.3	410	F

(Continued on p. 204)

TABLE II (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Chlormethiazole	0.23	0.1	–	–
Chloropyrilene	0.96	4.0	17	B
Chloroquine*	3.11	15.2	26	C
Chlorpheniramine	0.94	3.9	80	D
Chlorphenoxamine	0.80	2.9	530	G
Chlorphentermine	0.38	0.9	–	–
Chlorprenaline	0.41	1.1	210	F
Chlorproethazine	0.82	3.2	30	C
Chlorpromazine	0.98	4.1	30	C
Chlorprothixene	0.80	3.0	24	C
Cimetidine	0.27	0.4	200	E
Cinchocaine	0.56	1.9	66	D
Cinchonidine	0.80	3.1	100	D
Cinnarizine	0.36	0.8	22	C
Clemastine	0.89	3.7	740	G
Clemizole*	1.12	4.8	44	C
Clomipramine	0.85	3.4	67	D
Clonidine	0.45	1.2	330	F
Clonitazene	0.25	0.3	27	C
Cocaine	0.72	2.8	94	D
Codeine*	1.06	4.8	310	F
Colchicine	0.25	0.2	–	–
Cotarnine*	1.77	8.2	–	–
Cotinine	0.23	0.2	–	–
Cyclazocine	0.60	2.1	1000	G
Cyclizine	0.74	2.9	950	G
Cyclopentamine	0.52	1.7	#	H
Cyclopentolate*	0.49	1.6	1000	G
Cyproheptadine	0.86	3.2	30	C
Cyrenorphine	0.28	0.4	260	F
Debrisoquine	0.44	1.2	–	–
Deptropine*	1.20	5.0	110	E
Desacetylthymoxamine	0.67	2.3	960	G
Desalkyldisopyramide	0.54	1.8	4	A
Desalkylflurazepam	0.21	0.1	–	–
Deserpidine	0.28	0.4	28	C
Desethylamiodarone	0.53	1.8	11	B
Desipramine	0.56	2.1	70	D
Desmethyldesipramine	0.46	1.3	63	D
Desmethylnortriptyline	0.45	1.2	–	–
Desomorphine*	1.22	5.4	850	G
Dextromethorphan*	1.26	5.6	990	G
Dextromoramide	0.32	0.7	760	G
Dextropropoxyphene	0.55	1.9	1200	H
Dextrorphan*	1.09	4.7	1200	H
Diampromide	0.38	1.0	310	F
Diazepam	0.21	0.1	–	–
Diazoxide	0.22	0.1	–	–
Dibenzepin	0.72	2.8	46	C
Dicyclomine	0.40	1.1	#	H

TABLE II (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Diethazine	0.86	3.4	28	C
Diethylcarbamazine	0.47	1.4	#	H
Diethylpropion	0.54	1.7	36	C
Diethylthiambutene	0.57	2.0	18	B
Dihydrocodeine*	1.57	7.2	540	G
Dihydroergotamine	0.31	0.6	120	E
Dihydromorphine*	1.43	5.7	620	G
Dimenoxadole	0.48	1.6	500	F
Dimethindene	1.22	5.1	32	C
Dimethisoquin	0.64	2.2	87	D
Dimethothiazine	0.61	2.1	17	B
Dimethoxanate*	1.28	5.8	49	C
Dimethylthiambutene	0.67	2.6	23	C
Dioxaphetyl butyrate	0.27	0.3	540	G
Diphenhydramine	0.87	3.3	980	G
Diphenhydramine N-oxide*	0.43	1.1	–	–
Diphenoxylate	0.24	0.2	370	F
Diphenoxylic acid*	0.30	0.6	580	G
Diphenylpyraline*	0.89	3.3	360	F
Dipipanone	0.62	2.2	280	F
Diprenorphine	0.32	0.6	480	F
Dipyridamole	0.25	0.2	17	B
Disopyramide	0.67	2.4	46	C
Dopamine*	0.73	2.7	340	F
Dothiepin	0.84	3.2	50	C
Dothiepin S-oxide*	1.14	4.6	44	C
Doxapram	0.29	0.4	370	F
Doxepin	0.93	3.7	49	C
Doxylamine	1.11	4.4	88	D
Droperidol	0.31	0.6	57	D
Ecgonine	0.40	1.1	#	H
Embramine	0.80	3.0	480	F
Emepronium	1.19	5.2	–	–
Emetine*	1.61	7.1	180	E
Ephedrine	0.40	1.0	150	E
Ergocornine	0.26	0.4	43	C
Ergocristine	0.25	0.3	44	C
Ergocristinine	0.25	0.3	39	C
Ergocryptine	0.26	0.4	43	C
Ergometrine	0.26	0.4	49	C
Ergosine	0.25	0.3	43	C
Ergosinine	0.25	0.3	39	C
Ergotamine	0.29	0.4	53	D
Etafedrine	0.56	1.9	1300	H
Etamiphylline	0.43	1.2	72	D
Ethoheptazine	0.87	3.3	1800	H
Ethopropazine	0.69	2.4	20	B
Ethylmorphine*	0.93	3.7	280	F
Etonitazene	0.29	0.4	29	C
Etorphine	0.31	0.6	240	F

(Continued on p. 206)

TABLE II (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Etoxeridine	0.51	1.4	1300	H
Fencamfamin	0.46	1.3	480	F
Fenethazine	0.98	4.0	27	C
Fenfluramine	0.47	1.3	36	C
Fenoterol	0.35	0.7	520	G
Fentanyl	0.35	0.8	500	F
Flavoxate	0.63	2.2	27	C
Fluopromazine	0.80	2.7	23	C
Flupenthixol	0.44	1.2	40	C
Flupenthixol S-oxide	0.48	1.3	30	C
Fluphenazine	0.44	1.2	21	C
Flurazepam	0.51	1.3	32	C
Glycopyrronium*	0.84	3.2	–	–
Haloperidol	0.44	1.2	63	D
Halopyramine	0.98	4.2	37	C
Harmine	0.34	0.8	18	B
Heroin*	0.77	3.0	260	F
Histapyrrodine	0.76	3.0	39	C
Homatropine*	1.01	4.2	710	G
Hydrocodone*	1.68	7.1	270	F
Hydromorphinol*	0.85	3.1	750	G
Hydromorphone*	1.84	7.9	510	G
Hydroxypethidine*	0.69	2.3	850	G
Hydroxyzine	0.49	1.4	490	F
Hyoscine	0.42	1.1	940	G
Hyoscyamine*	0.90	3.7	500	F
Ibogaine	0.60	2.1	120	E
Imipramine	1.00	4.2	61	D
Imipramine N-oxide*	0.58	1.8	26	C
Indapamine	0.22	0.1	36	C
Indole	0.21	0.1	82	D
Iprindole	1.00	4.1	300	F
Isolysergide	0.67	2.6	52	D
Isomethadone	0.57	1.8	490	F
Isopropamide*	0.69	2.4	–	–
Isothipendyl	0.97	3.8	22	C
Isoxsuprine	0.36	0.8	230	F
Ketanserin	0.30	0.6	44	C
Ketobemidone*	0.78	2.8	790	G
Labetalol*	0.52	1.7	250	F
Laudanosine	0.97	4.1	110	E
Levallorphan*	0.58	1.9	630	G
Levomethorphan*	1.22	4.9	730	G
Levorphanol*	1.04	4.4	1300	H
Lidoflazine	0.31	0.6	240	F
Lignocaine	0.30	0.6	870	G
Lofepramine	0.32	0.6	36	C
Lorazepam	0.21	0.1	–	–
Lorcainide	0.58	1.8	310	F
Loxapine	0.41	1.1	47	C

TABLE II (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Lysergamide	0.31	0.5	68	D
Lysergic acid*	0.36	0.8	83	D
Lysergide (LSD)	0.37	0.7	42	C
Lysergol	0.40	1.1	64	D
Maprotiline	0.64	2.2	30	C
Mazindol	0.54	1.8	–	–
Mebanazine	0.24	0.2	13	B
Mebeverine	0.58	1.9	21	C
Mebhydrolin*	0.78	3.0	45	C
Mecamylamine	0.53	1.7	#	H
Meclophenoxate	0.53	1.7	530	G
Meclozine	0.33	0.7	220	F
Medazepam	0.23	0.2	15	B
Mepenzolate*	1.00	4.1	–	–
Mephenesin	0.24	0.2	–	–
Mephentermine	0.50	1.5	150	E
Mepivacaine	0.37	0.9	550	G
Meptazinol	0.79	3.1	1000	G
Mepyramine	0.96	3.9	24	C
Mequitazine*	1.87	8.3	100	D
Mescaline	0.47	1.3	13	B
Mesoridazine	1.17	5.0	26	C
Metaraminol	0.37	0.9	190	E
Metazocine*	1.06	4.1	900	G
Methadone	0.64	2.2	670	G
Methadone (Metabolite 1)	0.77	2.8	–	–
Methadone (Metabolite 2)	0.23	0.2	–	–
Methapyrilene	1.02	4.1	40	C
Methaqualone	0.25	0.2	–	–
Methdilazine	1.35	6.0	31	C
Methixine	0.91	3.6	74	D
Methocarbamol	0.22	0.1	–	–
Methoserpidine	0.29	0.5	24	C
Methotrimeprazine	0.83	3.2	20	B
Methoxamine	0.38	0.9	130	E
Methoxyphenamine	0.52	1.7	19	B
Methoxypromazine	1.17	5.2	29	C
Methylamphetamine	0.60	2.0	150	E
Methyldesorphine*	1.22	4.9	620	G
Methylephedrine	0.64	2.3	950	G
Methylergometrine	0.27	0.4	44	C
Methylphenidate	0.53	1.7	29	C
Methysergide	0.27	0.4	29	C
Metoclopramide	1.17	5.0	90	D
Metopimazine	0.47	1.4	19	B
Metoprolol	0.47	1.3	60	D
Mexiletine	0.43	1.2	–	–
Mianserin	0.54	1.8	150	E
Monoethylglycinexylidide (MEGX)	0.43	1.2	110	E
Morazone	0.33	0.7	42	C

(Continued on p. 208)

TABLE II (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Morpheridine	0.53	1.6	2800	H
Morphine N-oxide*	0.87	3.2	180	E
Morphine*	1.05	3.8	290	F
Myrophine*	0.90	3.3	220	F
Nadolol	0.43	1.2	59	D
Nalorphine	0.40	1.0	610	G
Naloxone	0.49	1.4	430	F
Naphazoline	0.68	2.4	8	A
Narceine	0.33	0.7	38	C
Nefopam	0.78	3.0	610	G
Neostigmine*	1.13	4.7	–	–
Nialamide*	0.43	1.2	39	C
Nicocodine*	0.89	3.7	89	D
Nicotine	0.42	1.1	110	E
Nifedipine	0.24	0.2	34	C
Nitrazepam	0.21	0.1	–	–
Nomifensine	0.37	0.9	130	E
Norbutriptyline	0.52	1.7	90	D
Norchlorpromazine	0.66	2.2	16	B
Norclomipramine	0.61	2.0	63	D
Norcodeine*	0.82	3.1	61	D
Norcyclizine	0.63	2.2	440	F
Nordextropropoxyphene	0.47	1.3	510	G
Nordiazepam	0.23	0.2	–	–
Nordothiepin	0.63	2.2	8	A
Nordothiepin S-oxide	0.84	3.1	7	A
Nordoxepin	0.63	2.2	14	B
Norfenfluramine	0.40	1.0	3	A
Normaprotiline	0.43	1.1	–	–
Normianserin	0.70	2.4	120	E
Normorphine*	0.78	2.9	160	E
Nororphenadrine	0.55	1.7	24	C
Norpethidine*	0.53	1.7	10	A
Norpseudoephedrine	0.39	1.0	–	–
Nortrimipramine	0.57	1.8	68	D
Nortriptyline	0.58	2.0	18	B
Norverapamil	0.51	1.7	180	E
Norzimelidine*	0.80	2.9	5	A
Noscapine	0.26	0.3	75	D
Opipramol	0.64	2.2	31	C
Orphenadrine	0.80	3.0	570	G
Orphenadrine N-oxide*	0.40	1.1	–	–
Oxeladin	0.80	3.0	1900	H
Oxprenolol	0.46	1.3	14	B
Oxycodone*	1.58	6.9	210	F
Oxymetazolin	0.52	1.7	190	E
Oxymorphone*	1.53	6.7	500	F
Oxypertine	0.33	0.7	62	D
Oxyphencyclimine	0.74	2.8	–	–
Oxyphenonium*	0.71	2.6	–	–

TABLE II (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
p-Chlorodisopyramide (CDP)	0.61	2.1	51	D
p-Methoxyamphetamine	0.43	1.1	–	–
Papaverine	0.27	0.3	36	C
Pargyline	0.23	0.2	480	F
Pecazine	0.97	3.9	22	C
Pemoline	0.24	0.2	–	–
Penbutolol	0.43	1.2	60	D
Pentazocine	0.53	1.8	760	G
Penthienate	0.86	3.2	2	A
Perhexiline	0.24	0.2	–	–
Pericyazine	0.46	1.3	23	C
Perphenazine	0.57	1.9	20	B
Pethidine*	0.72	2.8	1000	G
Pethidinic acid*	0.76	2.8	1700	H
Phenadoxone	0.30	0.4	470	F
Phenampromide	0.49	1.4	1000	G
Phenazocine	0.47	1.3	600	G
Phenazone	0.24	0.2	–	–
Phenbutrazate	0.26	0.3	630	G
Phencyclidine*	0.74	2.4	430	F
Phendimetrazine	0.36	0.9	2000	H
Phenelzine	0.39	1.0	1700	H
Phenglutarimide	0.80	2.9	630	G
Phenindamine	0.72	2.5	32	C
Pheniramine	1.00	4.1	76	D
Phenmetrazine	0.53	1.7	1200	H
Phenomorphan	0.51	1.4	890	G
Phenoperidine	0.37	0.8	280	F
Phenothiazine	0.22	0.1	11	B
Phenoxybenzamine	0.23	0.1	280	F
Phentermine	0.30	0.6	–	–
Phentolamine	0.55	1.7	94	D
Phenylephrine	0.48	1.3	540	G
Phenylpropanolamine	0.40	0.9	–	–
Phenyltoloxamine	0.84	3.1	78	D
Pholcodeine*	1.44	6.0	450	F
Physostigmine	0.71	2.6	28	C
Piminodine	0.40	1.0	93	D
Pimozide	0.34	0.7	330	F
Pindolol	0.40	1.2	38	C
Pipamazine	0.50	1.5	18	B
Pipazethate	1.32	5.4	54	D
Piperacetazine	0.55	1.9	31	C
Piperidolate	0.55	1.7	430	F
Pipradrol	0.44	1.2	14	B
Pirbuterol*	0.87	3.6	110	E
Pirenzepine	0.74	2.7	69	D
Piritramide	0.32	0.6	630	G
Pizotifen	0.91	3.4	40	C
Poldine*	0.89	3.3	–	–

(Continued on p. 210)

TABLE II (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Practolol	0.31	0.5	7	A
Prajmalium*	0.63	2.2	130	E
Pramoxine	0.32	0.6	1100	H
Prazosin	0.35	0.8	19	B
Prenylamine	0.40	1.0	300	F
Prilocaine	0.39	1.0	40	C
Primaquine	0.48	1.4	17	B
Proadifen	0.53	1.6	650	G
Procainamide	0.80	3.1	47	C
Procaine	0.60	1.9	160	E
Prochlorperazine	1.01	3.9	18	B
Procyclidine	0.60	2.0	1000	G
Proheptazine	0.86	3.2	2100	H
Prolintane	0.61	2.0	1200	H
Promazine	1.38	5.9	32	C
Promethazine	1.20	5.0	38	C
Pronethalol	0.45	1.3	34	C
Propantheline	1.11	4.4	–	–
Properidine	0.68	2.3	1500	H
Propiomazine	0.61	2.1	27	C
Propranolol	0.47	1.3	66	D
Prothipendyl	1.10	4.4	28	C
Protokylol*	0.79	3.1	270	F
Protriptyline	0.60	2.1	15	B
Proxymetacaine	0.64	2.1	78	D
Proxyphylline	0.22	0.1	–	–
Pseudoephedrine	0.42	1.2	12	B
Psilocin*	0.81	3.1	120	E
Pyridostigmine*	1.47	6.3	–	–
Pyrimethamine	0.38	1.0	9	A
Pyrrobutamine	0.76	2.8	29	C
Quinidine	0.64	2.1	75	D
Quinine	0.66	2.4	82	D
Ranitidine	0.68	2.3	69	D
Reproterol	0.43	1.2	130	E
Rescinnamine	0.32	0.6	48	C
Salbutamol	0.39	1.0	350	F
Sotalol	0.43	1.2	710	G
Strychnine*	2.74	13.0	67	D
Tacrine	0.49	1.6	56	D
Terazosin	0.40	1.1	8	A
Terbutaline	0.37	0.9	570	G
Terfenadine	0.39	1.0	290	F
Thebacon*	0.95	3.7	410	F
Thebaine*	1.06	4.6	51	D
Thenalidine	0.90	3.5	35	C
Thenyldiamine	0.96	4.0	33	C
Theobromine	0.23	0.1	–	–
Theophylline	0.23	0.1	52	D
Thiamine	0.58	2.0	–	–

TABLE II (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Thiethylperazine	0.96	3.8	26	C
Thiopropazate	0.40	1.0	19	B
Thioproperazine	1.01	4.1	27	C
Thioridazine	1.22	5.2	23	C
Thiothixene	0.96	3.8	40	C
Thonzylamine	0.84	3.2	56	D
Thymoxamine	0.79	2.9	380	F
Tigloidine*	0.91	3.6	180	E
Timolol	0.43	1.2	140	E
Tocainide	0.43	1.2	14	B
Tofenacin	0.54	1.7	27	C
Tolazoline	0.59	2.1	–	–
Tolpropamine	0.74	2.9	480	F
Tolycaine	0.32	0.7	66	D
Tramazoline	0.56	1.8	53	D
Tranylcypromine	0.40	1.0	1200	H
Trazodone	0.31	0.6	51	D
Trifluoperazine	0.83	3.0	26	C
Trifluperidol	0.44	1.2	38	C
Trimeperidine	0.63	2.1	1900	H
Trimeprazine	0.82	3.1	25	C
Trimetazidine*	0.82	3.0	670	G
Trimethobenzamide	1.09	4.7	20	B
Trimethoprim	0.43	1.2	43	C
Trimipramine	0.72	2.7	73	D
Tripelennamine	0.91	3.6	45	C
Triprolidine	0.86	3.2	29	C
Tryptamine	0.43	1.2	110	E
Tyramine	0.43	1.2	630	G
Verapamil	0.60	2.6	160	E
Viloxazine	0.72	2.7	380	F
Xylometazoline	0.52	1.6	8	A
Zimelidine*	0.83	3.2	15	B

detector settings. For a particular analysis, the detectors should be used under the most appropriate conditions and response ratios measured from injections of standard solutions. Note that stereoisomers are not resolved and therefore separate retention and response data have not been generated except in the case of commonly-encountered compounds such as quinine/quinidine. Analytes with k' values less than 1 will not normally be differentiated from non-retained co-extractives and thus alternative eluent conditions (different pH, lower ionic strength, etc.) should be used in the analysis of these compounds as appropriate.

The retention and relative response data in Tables II and III were obtained using a UV monitor fitted with a 10-μl volume, 8-mm path-length flow-cell connected in series with the electrochemical detector using 0.3 mm I.D. PTFE tubing. The retention times relative to imipramine were measured from reference injections performed with each batch of eluent. The detector response ratios were calculated to

TABLE III

RETENTION AND RESPONSE DATA (UV, 254 nm AND ELECTROCHEMICAL OXIDATION, +1.2 V) FOR THE COMPOUNDS STUDIED IN RELATIVE RETENTION TIME ORDER

For details, see legend to Table II.

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Desalkylflurazepam	0.21	0.1	–	–
Diazepam	0.21	0.1	–	–
Lorazepam	0.21	0.1	–	–
Indole	0.21	0.1	82	D
Nitrazepam	0.21	0.1	–	–
Phenothiazine	0.22	0.1	11	B
Methocarbamol	0.22	0.1	–	–
Diazoxide	0.22	0.1	–	–
Proxyphylline	0.22	0.1	–	–
Acetanilide	0.22	0.1	–	–
Acetazolamide	0.22	0.1	–	–
Indapamine	0.22	0.1	36	C
Methadone (Metabolite 2)	0.23	0.2	–	–
Benzocaine	0.23	0.1	43	C
Theophylline	0.23	0.1	52	D
Medazepam	0.23	0.2	15	B
Nordiazepam	0.23	0.2	–	–
Cotinine	0.23	0.2	–	–
Theobromine	0.23	0.1	–	–
Phenoxybenzamine	0.23	0.1	280	F
Bezitramide	0.23	0.2	43	C
Pargyline	0.23	0.2	480	F
Chlormethiazole	0.23	0.1	–	–
Phenazone	0.24	0.2	–	–
Mebanazine	0.24	0.2	13	B
Nifedipine	0.24	0.2	34	C
Perhexiline	0.24	0.2	–	–
Mephenesin	0.24	0.2	–	–
Diphenoxylate	0.24	0.2	370	F
Pemoline	0.24	0.2	–	–
Ergosinine	0.25	0.3	39	C
Colchicine	0.25	0.2	–	–
Ergocristinine	0.25	0.3	39	C
Caffeine	0.25	0.2	–	–
Clonitazene	0.25	0.3	27	C
Ergocristine	0.25	0.3	44	C
Methaqualone	0.25	0.2	–	–
Ergosine	0.25	0.3	43	C
Dipyridamole	0.25	0.2	17	B
Ergometrine	0.26	0.4	49	C
Ergocornine	0.26	0.4	43	C
Benzquinamide	0.26	0.3	260	F
Noscapine	0.26	0.3	75	D
Ergocryptine	0.26	0.4	43	C
Phenbutrazate	0.26	0.3	630	G
Acetorphine	0.27	0.4	210	F

TABLE III (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Cimetidine	0.27	0.4	200	E
Dioxaphetyl butyrate	0.27	0.3	540	G
Papaverine	0.27	0.3	36	C
Bromhexine	0.27	0.4	65	D
Methysergide	0.27	0.4	29	C
Methylergometrine	0.27	0.4	44	C
Amidopyrine	0.27	0.3	79	D
Deserpidine	0.28	0.4	28	C
Cyrenorphine	0.28	0.4	260	F
Buprenorphine	0.28	0.4	430	F
Ergotamine	0.29	0.4	53	D
Doxapram	0.29	0.4	370	F
Etonitazene	0.29	0.4	29	C
Methoserpidine	0.29	0.5	24	C
Phentermine	0.30	0.6	–	–
Ketanserin	0.30	0.6	44	C
Lignocaine	0.30	0.6	870	G
Diphenoxylic acid*	0.30	0.6	580	G
Phenadoxone	0.30	0.4	470	F
Lysergamide	0.31	0.5	68	D
Trazodone	0.31	0.6	51	D
Practolol	0.31	0.5	7	A
Etorphine	0.31	0.6	240	F
Lidoflazine	0.31	0.6	240	F
Dihydroergotamine	0.31	0.6	120	E
Droperidol	0.31	0.6	57	D
Dextromoramide	0.32	0.7	760	G
Piritramide	0.32	0.6	630	G
Lofepramine	0.32	0.6	36	C
Diprenorphine	0.32	0.6	480	F
Rescinnamine	0.32	0.6	48	C
Buclizine	0.32	0.7	490	F
Pramoxine	0.32	0.6	1100	H
Tolycaine	0.32	0.7	66	D
Narceine	0.33	0.7	38	C
Oxypertine	0.33	0.7	62	D
Morazone	0.33	0.7	42	C
Meclozine	0.33	0.7	220	F
Pimozide	0.34	0.7	330	F
Harmine	0.34	0.8	18	B
Fenoterol	0.35	0.7	520	G
Fentanyl	0.35	0.8	500	F
Prazosin	0.35	0.8	19	B
Cinnarizine	0.36	0.8	22	C
Lysergic acid*	0.36	0.8	83	D
Phendimetrazine	0.36	0.9	2000	H
Amphetamine	0.36	0.9	–	–
Isoxsuprine	0.36	0.8	230	F
Bupivacaine	0.36	0.9	670	G
Phenoperidine	0.37	0.8	280	F

(Continued on p. 214)

TABLE III (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Metaraminol	0.37	0.9	190	E
Buphenine	0.37	0.9	410	F
Terbutaline	0.37	0.9	570	G
Nomifensine	0.37	0.9	130	E
Lysergide (LSD)	0.37	0.7	42	C
Mepivacaine	0.37	0.9	550	G
Benzoylecgonine*	0.37	0.9	32	C
Bamethane	0.37	0.9	420	F
Chlorphentermine	0.38	0.9	–	–
Diampromide	0.38	1.0	310	F
Methoxamine	0.38	0.9	130	E
Pyrimethamine	0.38	1.0	9	A
Norpseudoephedrine	0.39	1.0	–	–
Prilocaine	0.39	1.0	40	C
Salbutamol	0.39	1.0	350	F
Terfenadine	0.39	1.0	290	F
Phenelzine	0.39	1.0	1700	H
Piminodine	0.40	1.0	93	D
Dicyclomine	0.40	1.1	#	H
Anileridine	0.40	1.1	95	D
Orphenadrine N-oxide*	0.40	1.1	–	–
Lysergol	0.40	1.1	64	D
Tranylcypromine	0.40	1.0	1200	H
Thiopropazate	0.40	1.0	19	B
Phenylpropanolamine	0.40	0.9	–	–
Norfenfluramine	0.40	1.0	3	A
Nalorphine	0.40	1.0	610	G
Pindolol	0.40	1.2	38	C
Terazosin	0.40	1.1	8	A
Ecgonine	0.40	1.1	#	H
Ephedrine	0.40	1.0	150	E
Prenylamine	0.40	1.0	300	F
Benperidol	0.41	1.1	97	D
3-Methoxy-4,5-methylenedioxyamphetamine	0.41	1.1	–	–
Loxapine	0.41	1.1	47	C
Chlorprenaline	0.41	1.1	210	F
Pseudoephedrine	0.42	1.2	12	B
Nicotine	0.42	1.1	111	E
Hyoscine	0.42	1.1	940	G
Normaprotiline	0.43	1.1	–	–
Etamiphylline	0.43	1.2	72	D
Mexiletine	0.43	1.2	–	–
Trimethoprim	0.43	1.2	43	C
Sotalol	0.43	1.2	710	G
Reproterol	0.43	1.2	130	E
p-Methoxyamphetamine	0.43	1.1	–	–
Tryptamine	0.43	1.2	110	E
Tyramine	0.43	1.2	630	G
Nadolol	0.43	1.2	59	D
Monoethylglycinexylidide (MEGX)	0.43	1.2	110	E

TABLE III (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Nialamide*	0.43	1.2	39	C
Azacyclonal	0.43	1.2	4	A
Diphenhydramine N-oxide*	0.43	1.1	–	–
Benzphetamine	0.43	1.2	600	G
Timolol	0.43	1.2	140	E
Tocainide	0.43	1.2	14	B
Penbutolol	0.43	1.2	60	D
4-Hydroxypropranolol	0.43	1.1	120	E
Pipradrol	0.44	1.2	14	B
Fluphenazine	0.44	1.2	21	C
Haloperidol	0.44	1.2	63	D
Debrisoquine	0.44	1.2	–	–
2-Phenylethylamine	0.44	1.2	–	–
Alprenolol	0.44	1.2	13	B
1-Phenylethylamine	0.44	1.2	–	–
Trifluperidol	0.44	1.2	38	C
Flupenthixol	0.44	1.2	40	C
2-Hydroxydesipramine	0.45	1.2	40	C
Atenolol	0.45	1.3	150	E
Clonidine	0.45	1.2	330	F
Pronethalol	0.45	1.3	34	C
Desmethylnortriptyline	0.45	1.2	–	–
Butacaine	0.45	1.2	120	E
Pericyazine	0.46	1.3	23	C
Bromperidol	0.46	1.3	46	C
Oxprenolol	0.46	1.3	14	B
Benzethidine	0.46	1.4	990	G
Fencamfamin	0.46	1.3	480	F
Desmethyldesipramine	0.46	1.3	63	D
Propranolol	0.47	1.3	66	D
Diethylcarbamazine	0.47	1.4	#	H
Mescaline	0.47	1.3	13	B
Fenfluramine	0.47	1.3	36	C
Metopimazine	0.47	1.4	19	B
Phenazocine	0.47	1.3	600	G
Nordextropropoxyphene	0.47	1.3	510	G
Metoprolol	0.47	1.3	60	D
Acebutolol	0.48	1.4	6	A
Primaquine	0.48	1.4	17	B
Dimenoxadole	0.48	1.6	500	F
Phenylephrine	0.48	1.3	540	G
Flupenthixol S-oxide	0.48	1.3	30	C
Cyclopentolate*	0.49	1.6	1000	G
Phenampromide	0.49	1.4	1000	G
Naloxone	0.49	1.4	430	F
Tacrine	0.49	1.6	56	D
Hydroxyzine	0.49	1.4	490	F
Mephentermine	0.50	1.5	150	E
Pipamazine	0.50	1.5	18	B
Etoxeridine	0.51	1.4	1300	H

(Continued on p. 216)

TABLE III (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Flurazepam	0.51	1.3	32	C
Phenomorphan	0.51	1.4	890	G
Benzoctamine	0.51	1.7	73	D
Norverapamil	0.51	1.7	180	E
Methoxyphenamine	0.52	1.7	19	B
Benactyzine	0.52	1.7	640	G
Oxymetazolin	0.52	1.7	190	E
Labetalol*	0.52	1.7	250	F
Norbutriptyline	0.52	1.7	90	D
Cyclopentamine	0.52	1.7	#	H
Xylometazoline	0.52	1.6	8	A
Proadifen	0.53	1.6	650	G
Mecamylamine	0.53	1.7	#	H
Methylphenidate	0.53	1.7	29	C
Norpethidine*	0.53	1.7	10	A
Meclophenoxate	0.53	1.7	530	G
Betameprodine	0.53	1.8	800	G
Desethylamiodarone	0.53	1.8	11	B
Morpheridine	0.53	1.6	2800	H
Pentazocine	0.53	1.8	760	G
Phenmetrazine	0.53	1.7	1200	H
Adiphenine	0.53	1.8	950	G
Tofenacin	0.54	1.7	27	C
Diethylpropion	0.54	1.7	36	C
Mianserin	0.54	1.8	150	E
Mazindol	0.54	1.8	–	–
Desalkyldisopyramide	0.54	1.8	4	A
Phentolamine	0.55	1.7	94	D
Benzhexol	0.55	1.8	410	F
Piperidolate	0.55	1.7	430	F
Piperacetazine	0.55	1.9	31	C
Nororphenadrine	0.55	1.7	24	C
Dextropropoxyphene	0.55	1.9	1200	H
Etafedrine	0.56	1.9	1300	H
Desipramine	0.56	2.1	70	D
Cinchocaine	0.56	1.9	66	D
Tramazoline	0.56	1.8	53	D
Butethamate	0.56	1.9	1600	H
Nortrimipramine	0.57	1.8	68	D
Perphenazine	0.57	1.9	20	B
Alverine	0.57	1.8	930	G
Diethylthiambutene	0.57	2.0	18	B
Allylprodine	0.57	2.0	810	G
Betacetylmethadol	0.57	2.0	570	G
Antazoline	0.57	1.8	72	D
Carphenazine	0.57	1.7	25	C
Isomethadone	0.57	1.8	490	F
Thiamine	0.58	2.0	–	–
Acetophenazine	0.58	1.9	43	C
Nortriptyline	0.58	2.0	18	B

TABLE III (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Levallorphan*	0.58	1.9	630	G
Alphacetylmethadol	0.58	1.7	440	F
10-Hydroxynortriptyline	0.58	1.8	15	B
Lorcainide	0.58	1.8	310	F
Imipramine N-oxide*	0.58	1.8	26	C
Mebeverine	0.58	1.9	21	C
Tolazoline	0.59	2.1	–	–
Verapamil	0.60	2.6	160	E
Amotriphene	0.60	2.0	14	B
Protriptyline	0.60	2.1	15	B
Cyclazocine	0.60	2.1	1000	G
Methylamphetamine	0.60	2.0	150	E
Amethocaine	0.60	2.0	360	F
Procaine	0.60	1.9	160	E
Procyclidine	0.60	2.0	1000	G
Ibogaine	0.60	2.1	120	E
Prolintane	0.61	2.0	1200	H
p-Chlorodisopyramide (CDP)	0.61	2.1	51	D
Norclomipramine	0.61	2.0	63	D
Dimethothiazine	0.61	2.1	17	B
Propiomazine	0.61	2.1	27	C
Dipipanone	0.62	2.2	280	F
Alphamethadol	0.62	2.1	540	G
Norcyclizine	0.63	2.2	440	F
Trimeperidine	0.63	2.1	1900	H
Prajmalium*	0.63	2.2	130	E
Nordothiepin	0.63	2.2	8	A
Nordoxepin	0.63	2.2	14	B
Flavoxate	0.63	2.2	27	C
Betamethadol	0.63	2.3	370	F
Maprotiline	0.64	2.2	30	C
Quinidine	0.64	2.1	75	D
Methylephedrine	0.64	2.3	950	G
Dimethisoquin	0.64	2.2	87	D
Proxymetacaine	0.64	2.1	78	D
Opipramol	0.64	2.2	31	C
Methadone	0.64	2.2	670	G
Alphameprodine*	0.65	2.4	1100	H
Norchlorpromazine	0.66	2.2	16	B
Quinine	0.66	2.4	82	D
Amiodarone	0.66	2.4	23	C
Betaprodine	0.67	2.6	940	G
Dimethylthiambutene	0.67	2.6	23	C
Isolysergide	0.67	2.6	52	D
Disopyramide	0.67	2.4	46	C
Desacetylthymoxamine	0.67	2.3	960	G
Naphazoline	0.68	2.4	8	A
Properidine	0.68	2.3	1500	H
Chlorcyclizine	0.68	2.3	410	F
Ranitidine	0.68	2.3	69	D

(Continued on p. 218)

TABLE III (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Isopropamide*	0.69	2.4	–	–
Ethopropazine	0.69	2.4	20	B
Hydroxypethidine*	0.69	2.3	850	G
Normianserin	0.70	2.4	120	E
Physostigmine	0.71	2.6	28	C
Oxyphenonium*	0.71	2.6	–	–
Dibenzepin	0.72	2.8	46	C
Viloxazine	0.72	2.7	380	F
Butriptyline	0.72	2.7	890	G
Phenindamine	0.72	2.5	32	C
Trimipramine	0.72	2.7	73	D
Pethidine*	0.72	2.8	1000	G
Ajmaline*	0.72	2.8	110	E
Cocaine	0.72	2.8	94	D
Dopamine*	0.73	2.7	340	F
Alphaprodine*	0.74	2.8	1300	H
Oxyphencyclimine	0.74	2.8	–	–
Tolpropamine	0.74	2.9	480	F
Cyclizine	0.74	2.9	950	G
Phencyclidine*	0.74	2.4	430	F
Pirenzepine	0.74	2.7	69	D
Bromodiphenhydramine	0.75	2.7	420	F
Histapyrrodine	0.76	3.0	39	C
Pyrrobutamine	0.76	2.8	29	C
Pethidinic acid*	0.76	2.8	1700	H
11-Hydroxyclomipramine	0.76	2.9	50	C
Heroin*	0.77	3.0	260	F
Methadone (Metabolite 1)	0.77	2.8	–	–
Bufotenine	0.78	3.1	180	E
Normorphine*	0.78	2.9	160	E
Mebhydrolin*	0.78	3.0	45	C
N-Acetylprocainamide (NAPA)	0.78	3.0	24	C
Ketobemidone*	0.78	2.8	790	G
Nefopam	0.78	3.0	610	G
Thymoxamine	0.79	2.9	380	F
Protokylol*	0.79	3.1	270	F
Meptazinol	0.79	3.1	1000	G
Norzimelidine*	0.80	2.9	5	A
Fluopromazine	0.80	2.7	23	C
Chlorphenoxamine	0.80	2.9	530	G
Oxeladin	0.80	3.0	1900	H
Phenglutarimide	0.80	2.9	630	G
Cinchonidine	0.80	3.1	100	D
Embramine	0.80	3.0	480	F
Orphenadrine	0.80	3.0	570	G
Chlorprothixene	0.80	3.0	24	C
10-Hydroxyamitriptyline	0.80	2.9	37	C
Procainamide	0.80	3.1	47	C
Psilocin*	0.81	3.1	120	E
Chlorproethazine	0.82	3.2	30	C

TABLE III (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
3-Monoacetylmorphine*	0.82	3.1	400	F
Trimeprazine	0.82	3.1	25	C
Trimetazidine*	0.82	3.0	670	G
Norcodeine*	0.82	3.1	61	D
Betahistine	0.82	3.1	5	A
Amitriptyline	0.83	3.3	52	D
Trifluoperazine	0.83	3.0	26	C
Zimelidine*	0.83	3.2	15	B
2-Hydroxyimipramine	0.83	3.1	67	D
Methotrimeprazine	0.83	3.2	20	B
Thonzylamine	0.84	3.2	56	D
Glycopyrronium*	0.84	3.2	–	–
Nordothiepin S-oxide	0.84	3.1	7	A
Phenyltoloxamine	0.84	3.1	78	D
Dothiepin	0.84	3.2	50	C
Hydromorphinol*	0.85	3.1	750	G
Clomipramine	0.85	3.4	67	D
Triprolidine	0.86	3.2	29	C
Penthienate	0.86	3.2	2	A
Diethazine	0.86	3.4	28	C
Proheptazine	0.86	3.2	2100	H
Cyproheptadine	0.86	3.2	30	C
Ethoheptazine	0.87	3.3	1800	H
Morphine N-oxide*	0.87	3.2	180	E
Diphenhydramine	0.87	3.3	980	G
Pirbuterol*	0.87	3.6	110	E
Nicocodine*	0.89	3.7	89	D
Apomorphine*	0.89	3.7	82	D
Diphenylpyraline*	0.89	3.3	360	F
Poldine*	0.89	3.3	–	–
Clemastine	0.89	3.7	740	G
10-Hydroxyimipramine	0.90	3.4	41	C
Hyoscyamine*	0.90	3.7	500	F
Myrophine*	0.90	3.3	220	F
Thenalidine	0.90	3.5	35	C
Methixine	0.91	3.6	74	D
Tripelennamine	0.91	3.6	45	C
Tigloidine*	0.91	3.6	180	E
6-Monoacetylmorphine*	0.91	3.6	410	F
Pizotifen	0.91	3.4	40	C
Ethylmorphine*	0.93	3.7	280	F
Doxepin	0.93	3.7	49	C
Brompromazine	0.93	3.7	22	C
Chlorpheniramine	0.94	3.9	80	D
Benztropine*	0.94	3.7	120	E
Atropine*	0.94	3.9	650	G
Butaperazine	0.95	3.4	27	C
Thebacon*	0.95	3.7	410	F
Thiothixene	0.96	3.8	40	C
Thiethylperazine	0.96	3.8	26	C

(Continued on p. 220)

TABLE III (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Mepyramine	0.96	3.9	24	C
Chloropyrilene	0.96	4.0	17	B
Thenyldiamine	0.96	4.0	33	C
Isothipendyl	0.97	3.8	22	C
Laudanosine	0.97	4.1	110	E
Pecazine	0.97	3.9	22	C
Halopyramine	0.98	4.2	37	C
Brompheniramine	0.98	4.1	75	D
Fenethazine	0.98	4.0	27	C
Chlorpromazine	0.98	4.1	30	C
Imipramine	1.00	4.2	61	D
Iprindole	1.00	4.1	300	F
Mepenzolate*	1.00	4.1	–	–
Pheniramine	1.00	4.1	76	D
Homatropine*	1.01	4.2	710	G
Prochlorperazine	1.01	3.9	18	B
Thioproperazine	1.01	4.1	27	C
Acepromazine	1.02	4.1	42	C
Methapyrilene	1.02	4.1	40	C
Benzylmorphine*	1.03	4.4	210	F
Levorphanol*	1.04	4.4	1300	H
Morphine*	1.05	3.8	290	F
Thebaine*	1.06	4.6	51	D
Metazocine*	1.06	4.1	900	G
Codeine*	1.06	4.8	310	F
Dextrorphan*	1.09	4.7	1200	H
Trimethobenzamide	1.09	4.7	20	B
Bretylium*	1.09	4.3	–	–
Prothipendyl	1.10	4.4	28	C
Doxylamine	1.11	4.4	88	D
Propantheline	1.11	4.4	–	–
Clemizole*	1.12	4.8	44	C
Neostigmine*	1.13	4.7	–	–
Dothiepin S-oxide*	1.14	4.6	44	C
Carbinoxamine*	1.16	4.7	80	D
Methoxypromazine	1.17	5.2	29	C
Metoclopramide	1.17	5.0	90	D
Mesoridazine	1.17	5.0	26	C
Emepronium	1.19	5.2	–	–
Promethazine	1.20	5.0	38	C
Deptropine*	1.20	5.0	110	E
Desomorphine*	1.22	5.4	850	G
Levomethorphan*	1.22	4.9	730	G
Thioridazine	1.22	5.2	23	C
Methyldesorphine*	1.22	4.9	620	G
Dimethindene	1.22	5.1	32	C
Dextromethorphan*	1.26	5.6	990	G
Dimethoxanate*	1.28	5.8	49	C
Pipazethate	1.32	5.4	54	D
Methdilazine	1.35	6.0	31	C

TABLE III (*continued*)

Compound	*Relative retention time*	*k'*	*Detector response ratio*	
			Numeric	*Code*
Promazine	1.38	5.9	32	C
Dihydromorphine*	1.43	5.7	620	G
Pholcodeine*	1.44	6.0	450	F
Pyridostigmine*	1.47	6.3	–	–
Oxymorphone*	1.53	6.7	500	F
Dihydrocodeine*	1.57	7.2	540	G
Oxycodone*	1.58	6.9	210	F
Emetine*	1.61	7.1	180	E
Hydrocodone*	1.68	7.1	270	F
Cephaline*	1.68	7.7	340	F
Cotarnine*	1.77	8.2	–	–
Hydromorphone*	1.84	7.9	510	G
Mequitazine*	1.87	8.3	100	D
Brucine*	2.34	11.1	45	C
Strychnine*	2.74	13.0	67	D
Chloroquine*	3.11	15.2	26	C

two significant figures from peak height measurements obtained at sensitivity settings of 5 μA and 0.1 a.u. f.s.d., respectively. It is of course likely that slight differences in response ratios will be obtained using different detector configurations, although independent measurements in our two laboratories have shown good agreement for a large number of compounds. It is also possible that different batches of the glassy-carbon electrode material may show different response characteristics to those reported here. However, the response ratio codes (Tables II and III) give an indication of the magnitude of the ratio that should be obtained. On a given system the short-term variation in retention and response ratio was small and the long-term variation generally greater but not unacceptable (Table IV). However, the long-term variation in the response ratio of nortriptyline (secondary aliphatic amine) was large and is attributable to electrode deactivation as discussed previously. Whether the use of different solvent/ionic modifier combinations or of different electrode materials may help here remains to be seen.

The retention data presented in Tables II and III were obtained using one column (Spherisorb S5W, Batch No. 1651). Different batches of this material may give slight differences in absolute retention, although differences in retention relative to imipramine are less. Independent measurements performed in our two laboratories using columns packed with material from different batches again gave good agreement for a large number of compounds. The peak shape given for the same analyte may also differ between batches of material as well as between columns. The compounds giving rise to markedly tailing peaks on the column used in this work are indicated with asterisks in Tables II and III, the peak shape given by certain catecholamines and long-retained alkaloids being especially poor. The retention and response data for such compounds will necessarily be less reliable than for the other compounds studied. Whether the use of different counter-ions, solvents or station-

TABLE IV

INTER- AND INTRA-ASSAY VARIATIONS IN RETENTION AND RESPONSE RATIO (ELECTROCHEMICAL, +1.2 V AND UV, 254 nm) FOR SOME TEST COMPOUNDS

See legend to Fig. 3 for chromatographic conditions.

Analyte	*k'**			*Relative retention***			*Response ratio****		
		C.V. (%)§			*C.V. (%)*			*C.V. (%)*	
		(a)	*(b)*		*(a)*	*(b)*		*(a)*	*(b)*
Amphetamine	0.9	0.3	10.0	0.36	0.4	4.6	–	–	–
Nortriptyline	2.0	0.1	6.9	0.58	0.2	4.7	18.4	1.5	40
Amitriptyline	3.3	0.2	4.8	0.83	0.1	1.4	52.1	1.4	12
Imipramine	4.2	0.3	4.3	1.00	–	–	61.4	0.9	15
Methdilazine	6.0	0.2	3.9	1.35	0.1	1.0	30.9	1.5	9.7

* Column capacity factor.
** Retention relative to imipramine.
*** Electrochemical: UV (μA/a.u.).
§ C.V. = coefficient of variation (n = 10 in each case): (a), intra-assay; (b), inter-assay.

ary-phase materials may resolve this problem while retaining the advantages of silica column/non-aqueous ionic eluents is a topic for further study.

Although compounds such as flurazepam and quinine give very badly tailing peaks under strongly acidic conditions, others, notably those containing carboxylic acid or one or more phenolic hydroxyl functions, give better peaks. A strongly acidic eluent is of course mandatory in the analysis of very weak bases such as chlormethiazole and most benzodiazepines which are not retained at higher pHs. On the other hand, quinine and other alkaloids such as morphine are best analysed at a higher eluent pH such as 8.3[2]. The background current will be higher at +1.2 V at this pH, but either a lower detection potential can be employed or a proportion (*ca.* 10% v/v) of chloroform may be used in the eluent. Both approaches reduce the background current, although the addition of chloroform may give rise to changes in elution sequence and may preclude the simultaneous use of UV detection. Alternatively, a methanol–aqueous ammonium nitrate, pH 10.1 (9:1 v/v) eluent may be used, and retention data for a number of compounds on Syloid 74 silica[5,13,14], μPorasil[15] and Spherisorb S5W[16]are available. However, as noted previously this may restrict the applicability of electrochemical detection. Some factors influencing retention when using silica column/non-aqueous ionic eluents have been discussed previously[2] and should prove useful in the evaluation of different analytical systems.

In changing from strongly acidic to neutral eluent pHs and *vice versa*, stable retention times and electrochemical responses will only be obtained after appropriate equilibration of the column. For example, after equilibration of a 250 mm Spherisorb S5W silica column under strongly acidic conditions (0.05% v/v perchloric acid in methanol), 230 ml methanolic ammonium perchlorate (10 mM, pH 6.7) were required to "neutralise" the column as measured by the change in the background current of the electrochemical detector at +1.2 V. Use of a high ionic strength eluent, *e.g.* 0.1 M, before reverting to the normal eluent is a convenient means of giving rapid equil-

ibration when eluent pH changes are needed. Experience suggests that the eluent ionic strength required to promote elution under strongly acidic conditions decreases after prolonged use but that this effect does not occur using an ammonium perchlorate, pH 6.7 eluent. Thus, eluent pH changes of the type discussed above may also be useful in restoring full retentive properties to certain columns.

Metabolism of basic drugs often proceeds via N-dealkylation or aromatic hydroxylation thus giving compounds ideally suited for analysis using non-aqueous ionic eluent systems. N-dealkylated metabolites, for example, usually have shorter retention times than the parent compound at pH 6.7 and show similar UV characteristics but have a reduced electrochemical response at applied potentials less than 1.2 V (Fig. 10). Sulphoxides, particularly those of phenothiazines, are longer retained than the parent compound and show no electrochemical response below 0.8 V applied. On the other hand, phenolic hydroxyl metabolites are often shorter retained and may show enhanced electrochemical activity at lower applied potentials.

As with UV detection, the electrochemical detector gives a linear response over at least a thousand-fold range of analyte concentrations. In addition to providing information to aid in qualitative work, the electrochemical/UV response ratios (Table II) give an indication of the applicability of electrochemical detection to a particular compound. However, it should be remembered that analytes such as most phenothiazines which show good absorption at 254 nm give relatively low electrochemical/UV ratios (*ca.* 20–30) under the conditions used despite having excellent absolute electrochemical responses. On the other hand, ecgonine gives a relatively poor electrochemical response yet has negligible absorption at 254 nm, thus giving a high response ratio (Table II). Addition of a benzoyl moiety (benzoylecgonine, Table II)

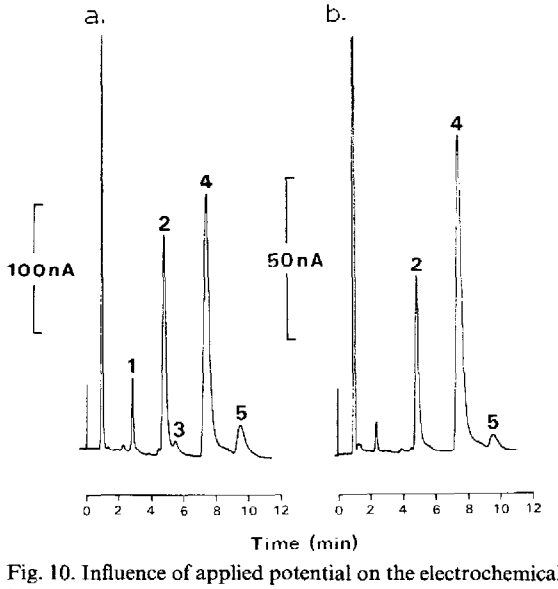

Fig. 10. Influence of applied potential on the electrochemical detection of dothiepin and some metabolites. Injection, 20 μl of concentrated extract of whole blood specimen (0.5 ml) from a patient known to have been taking dothiepin. Detection: electrochemical oxidation, (a) +1.2 V, (b) +1.0 V. Peaks: (1) nordothiepin, (2) dothiepin, (3) nordothiepin S-oxide, (4) methdilazine (internal standard), (5) dothiepin S-oxide. See legend to Fig. 3 for chromatographic conditions.

is here sufficient to alter the response ratio completely. UV absorption data from methanolic or ethanolic solution are often available in standard texts[17] and this together with knowledge of the electrochemical response of different oxidisable groups at different applied potentials (Fig. 3) should also assist in the choice of detection conditions for a particular analyte.

CONCLUSIONS

The use of an unmodified silica column with a non-aqueous ionic eluent gives a simple, reliable and flexible system for the analysis of a wide range of basic compounds. Efficient performance can be obtained for most analytes under appropriate eluent conditions, while the effect of alterations in a number of variables can be predicted from knowledge of some of the factors influencing retention. In practice, three eluents (0.02% v/v methanolic perchloric acid, and 10 m*M*, pH 6.7 and 10 m*M*, pH 8.3 methanolic ammonium perchlorate) have proved adequate for most applications. Furthermore, use of the pH 6.7 eluent together with serial UV/electrochemical detection gives a simple isocratic system which can be used in high-sensitivity qualitative "screening".

The availability of a variety of chemically bonded stationary phase materials suggests that they should be investigated for use with non-aqueous ionic eluents. Initial studies with amiodarone and a variety of dealkylated and deiodinated analogues showed that use of Spherisorb S5ODS1 gave some changes in elution sequence when compared to the results obtained using unmodified silica[1]. The use of stationary phase moieties such as propylsulphonic acid which should possess greater affinity for basic analytes than silica silanols, may permit the extension of the technique to very weak bases such as lorazepam which cannot be retained satisfactorily using unmodified silica. However, a potential disadvantage is that acidic/neutral compounds may be retained.

Finally, although reference has been made throughout to basic drugs and quaternary ammonium compounds, the system described is equally applicable to other basic organic compounds. Electrochemical detection may be especially useful in the analysis of aliphatic amines which are difficult to analyse by gas chromatography and which lack useful UV absorbance or fluorescence properties thus limiting the sensitivity previously attainable using HPLC.

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