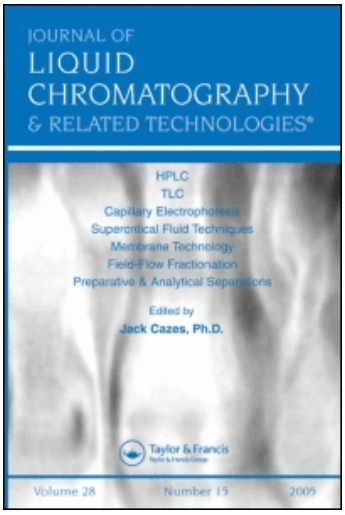

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MEASUREMENT OF CARDIOACTIVE DRUGS IN BIOLOGICAL SAMPLES BY HPLC

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ABSTRACT

Unmodified silica columns used with non-aqueous, usually methanolic, eluents containing an ionic modifier provide simple, flexible systems for the HPLC analysis of a wide range of basic cardioactive drugs and some quaternary ammonium compounds. Retention and peak shape are influenced primarily by eluent pH and ionic strength although changes in eluent composition and in the stationary phase may also prove useful. Recently it has been found that chemically-bonded strong cation exchange materials provide enhanced retention for analytes which are poorly retained on silica alone. Solvent extracts and elution fractions from bonded-phase extraction tubes may be analysed directly and, in addition to low-wavelength UV and fluorescence, electrochemical oxidation detection may be used routinely for a wide range of analytes including secondary and tertiary aliphatic amines.

INTRODUCTION

The measurement of cardioactive drugs and any pharmacologically active metabolites in biological specimens, most frequently plasma/serum, is

important in the evaluation of new compounds, in the optimisation of therapy and in the diagnosis of poisoning (1). For clinical use simple, rapid, selective assays are required which are able to cope with single 'urgent' analyses as well as 'batched' specimens. Sample requirements should be minimal (of the order of 20-200 ul) since multiple tests may be needed and the volume of specimen available may be limited, for example from neonates and young children.

High-performance liquid chromatography (HPLC) using chemically-bonded stationary phase materials and aqueous methanol or acetonitrile eluents has been employed widely (2-4). However, in addition to buffer salts, various pairing- or counter-ions are needed in order to give efficient performance for basic compounds. Furthermore, evaporation of solvent extracts and re-constitution in an aqueous medium is usually required prior to the analysis.

Simple solvent extractions with direct analysis of the extract have been used successfully in our laboratory for many years to measure a variety of compounds by gas-liquid chromatography (5,6). Accordingly, an attempt was made to develop analogous HPLC methodology. It was found that methanol containing an ionic modifier such as perchloric acid (0.01 or 0.02 % v/v, ca. 1 or 2 mM) gave efficient performance for a variety of basic drugs using unmodified silica columns while permitting the direct analysis of solvent extracts (7,8).

The aim of this paper is to review the approach to the HPLC of cardio-active basic drugs and quaternary ammonium compounds adopted in our laboratory and to introduce some recent findings.

PRACTICAL USE OF NON-AQUEOUS IONIC ELUENT/SILICA COLUMN SYSTEMS

1. Factors Influencing Retention and Peak Shape

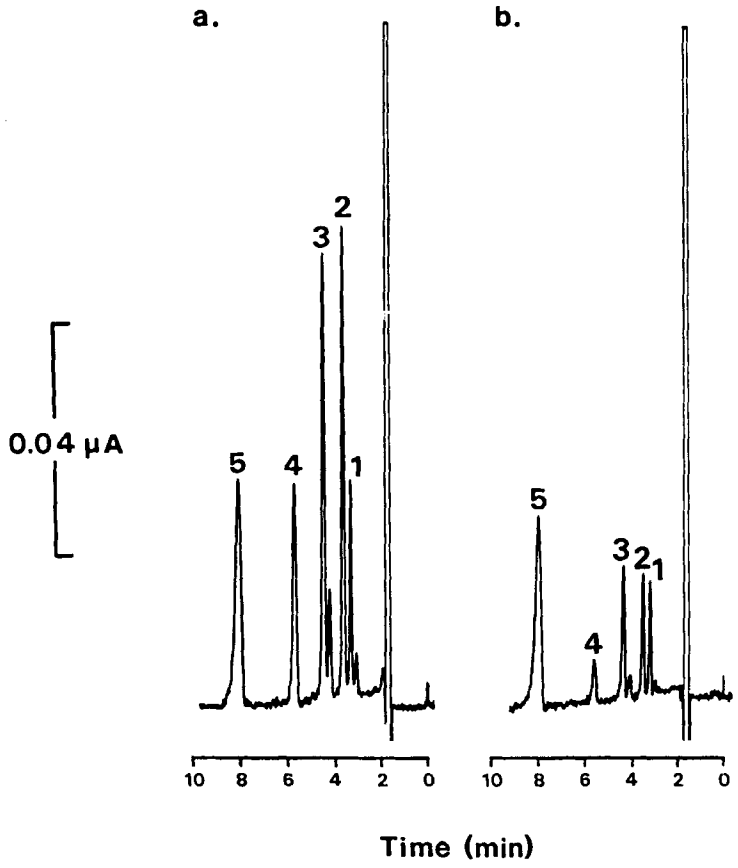
1.1. pH and Ionic Strength

It is likely that the retention of protonated bases and quaternary ammonium compounds on unmodified silica columns is mediated largely via cation-exchange with surface silanols. Thus, eluent pH and ionic strength are major influences on retention, the pKa often indicating the pH of maximum retention. The peak shape of certain analytes, notably alkaloids such as quinine/quinidine and morphine, is also affected by eluent pH although the reason for this is unclear (9). Eluent pH measurements performed directly using a glass electrode calibrated against aqueous buffers, although not corrected for the absence of water, can give practically useful information when fault-finding or developing new methods. When changing pH across columns it is important to allow adequate time for equilibration due to the buffering effect of silica, especially when changing from strongly acidic to neutral conditions and *vice versa*.

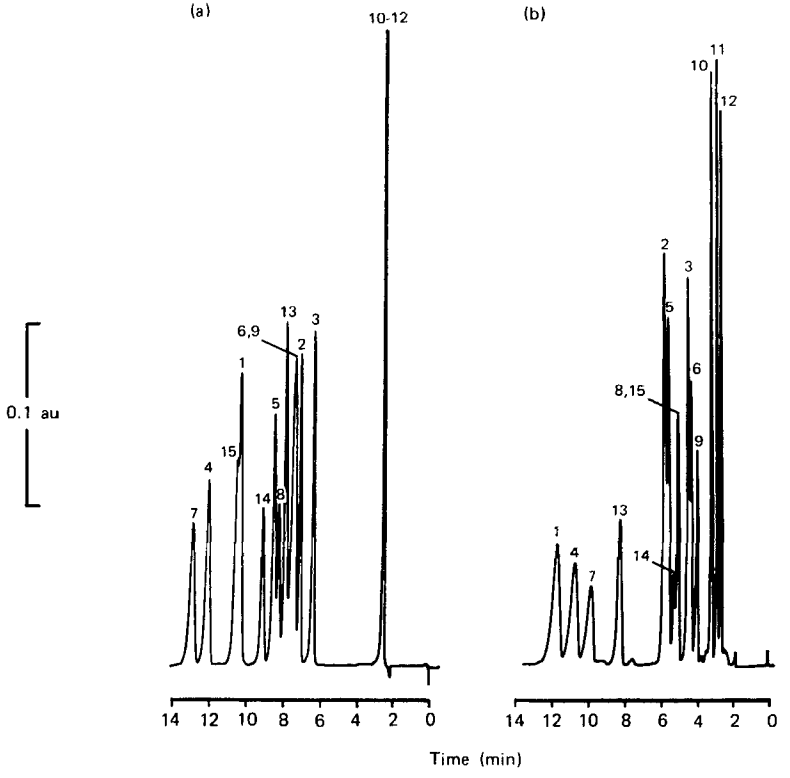
In practice, perchloric acid has proved a valuable modifier when a strongly acidic eluent is required while an ammonium perchlorate-modified eluent (10 mM, apparent pH 6.7) gives a compromise between retention, peak shape and electrochemical response (Section 2.2) for many analytes (10). Perchlorates are useful modifiers since they are adequately soluble in methanol, have virtually no UV absorption above 200 nm and are strongly resistant to oxidation. The ammonium ion

provides buffering capacity at neutral and alkaline pH values and the use of ammonium salts avoids the inaccuracies inherent in the use of free ammonia in eluent preparation. However, care may be needed to prevent the accumulation of potentially explosive perchlorate residues and camphorsulphonic acid provides a readily available alternative to perchloric acid. No problems with corrosion have been encountered in over 8 years operation other than on sporadic occasions with stainless steel tubing associated with pressure transducers in 750 series pumps (Applied Chromatography Systems), a problem not confined to non-aqueous ionic eluent applications.

Silica column/non-aqueous ionic eluent systems are especially suitable for basic drug analyses since N-dealkylated, aromatic hydroxyl or other metabolites are often resolved. The separation of verapamil, norverapamil and up to 4 additional metabolites in plasma extracts gives a good example here (Fig. 1). Note that the mobile phase used initially in the assay of verapamil (11) and also an eluent used for the assay of amiodarone in the presence of diazepam (12) contained potassium bromide as the ionic modifier. The use of this latter compound has now been abandoned since it has no buffering capacity, leading to unstable retention times at neutral or alkaline eluent pHs, and the bromide anion has a UV cutoff at ca. 220 nm. Finally, the separation of compounds with very similar structures which can be achieved using a semi-preparative (8 mm i.d.) column is illustrated by the analysis of a synthetic mixture containing amiodarone and a number of de-alkylated, de-iodinated and other analogues (Fig. 2). Of these, only amiodarone and desethylamidarone are encountered in extracts of samples from patients treated with amiodarone (13).



1. Analysis of verapamil in plasma. Column: 250 x 5 mm i.d. Spherisorb S5W silica. Eluent: Methanolic ammonium perchlorate (7 mM) adjusted to apparent pH 1.3 with methanolic perchloric acid (0.1 M). Flow-rate: 2 ml/min. Detection: fluorescence, excitation 203 nm, no emission filter (Kratos-Schoeffel FS 970). Injection: 100 ul of methyl tert.- butyl ether extract (pH 14) of plasma (100 ul). Specimens: (a) Pre- dose sample from patient prescribed 3 x 40 mg verapamil/day (verapamil & norverapamil concentrations 72 & 112 ug/l, respectively) and (b) 2 h sample from pregnant patient prescribed 2 x 80 mg daily (verapamil & norverapamil concentrations 10 & 15 ug/l, respectively). Peaks: 1 = D620 (verapamil metabolite), 2 = norverapamil, 3 = D617 (verapamil metabolite), 4 = verapamil, 5 = benzoquinoline (internal standard).



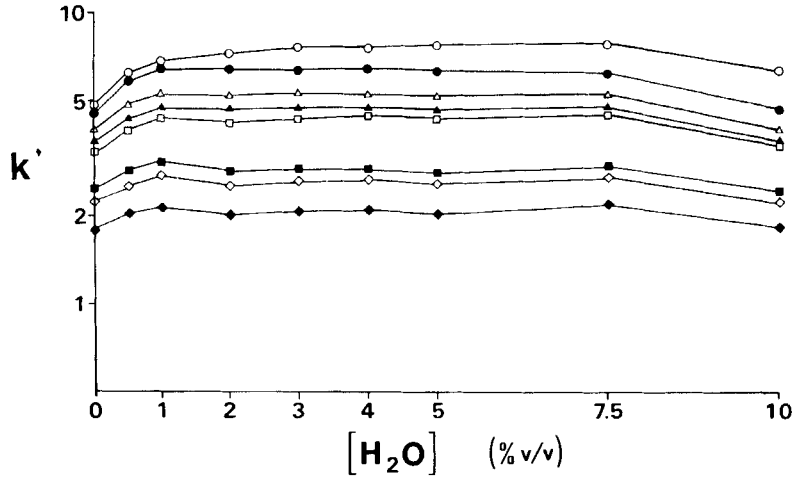
2. Separation of amiodarone and some dealkylated, de-iodinated & other analogues on 250 x 8 mm i.d. columns. Injection: 100 ul of methanolic solution containing ca. 10 mg/l of each compound. Detection: UV, 240 nm. Flow-rate: 4 ml/min. (a) Column: Spherisorb S5W silica, eluent: methanolic perchloric acid (0.04 % v/v). (b) Column: Spherisorb S5 ODS1, eluent: methanolic perchloric acid (0.02 % v/v). Peaks: 1 = amiodarone, 2 = desethylamiodarone, 3 = didesethylamiodarone, 4 = desiodoamiodarone, 7 = didesiodoamiodarone (see Ref. 13 for others).

Studies of the effect of eluent pH on retention suggest that quaternary ammonium compounds may be analysed selectively using a relatively high pH eluent (11 or above) since potential interference from basic drugs is minimised (9). However, a similar compound would be required as an internal standard and the only cardioactive quaternary ammonium compound studied, prajmalium, gave a relatively poor peak shape with an alkaline (pH 11) eluent and is best analysed at an acidic pH with fluorescence detection. On the other hand, compounds such as quinidine and procainamide give tailing peaks under acidic and neutral conditions and are best analysed at pH 8-9.

1.2. Eluent Solvent Composition

Minor alterations of eluent solvent composition rarely give rise to marked changes in retention or selectivity even with water under strongly acidic (Fig. 3) or neutral (9) conditions. However, codeine and morphine may be resolved at pH 9.2 using methanol:chloroform (40:60) rather than methanol alone (8) and penbutolol and 4-hydroxy-penbutolol can be similarly resolved using a high proportion (75% v/v) of iso-octane/methyl tert.-butyl ether and 25% methanol in an ammonium perchlorate-modified eluent (14).

We have often added a proportion (ca. 20% v/v) of a relatively non-polar solvent such as n-hexane, iso-octane (2,2,4-trimethylpentane) or methyl tert.-butyl ether to certain eluents since it was felt that this might minimise the risk of accumulation of methanol-insoluble residues. However, it is doubtful if such additions markedly influence retention or selectivity. Similarly, when analysing methyl tert.-butyl



3. Influence of eluent water content on the chromatography of some test compounds. Column: 250 x 5 mm i.d. Spherisorb S5W silica. Eluent: perchloric acid (0.02 % v/v) in methanol:water. Flow-rate: 2 ml/min. Detection: UV, 254 nm. Injection: 20 ul of methanolic solutions (10 mg/l except amphetamine & emepronium, 100 mg/l). Key: ◆ = amphetamine, ◇ = desethylamiodarone, ■ = nortriptyline, □ = amiodarone, ▲ = amitriptyline, △ = imipramine, ● = methdilazine, ○ = emepronium.

ether sample extracts directly, eluent recirculation does not cause alterations in chromatographic behaviour unless such recirculation is prolonged, in which case some decrease in retention may occur.

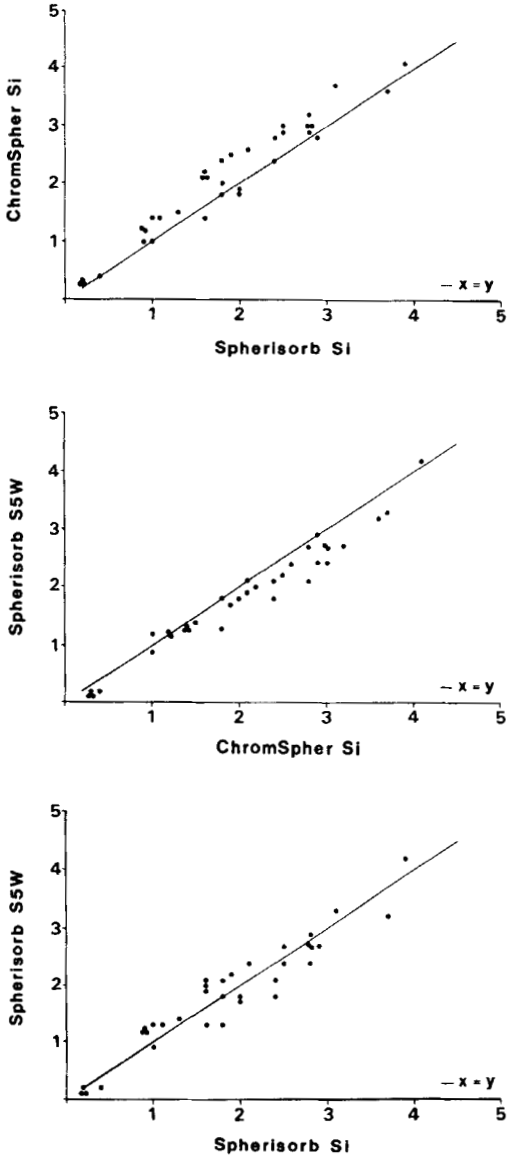
1.3. Nature of the Stationary Phase

We use Spherisorb S5W silica (5u average particle size) routinely but similar results may be obtained using other unmodified silicas (9). Differences in retention and, more especially, peak shape for certain analytes have been noted between different Spherisorb S5W columns although such differences rarely preclude the use of a column

for a particular analysis. We have found little advantage to the use of 3u materials such as Spherisorb S3W silica although this may be because specialised low dead-volume equipment would be needed to take full advantage of the enhanced separating power available. ChromSep^R (Chrompack-Packard) pre-packed glass cartridges (3 mm i.d.) give similar performance (retention and peak shape) to conventional 5 mm i.d. stainless steel columns but at lower flow-rates. A comparison of retention data for 32 compounds (cardioactive drugs and some other commonly-occurring compounds such as tricyclic antidepressants) on cartridge columns packed with 'Spherisorb Si' (15) and ChromSpher Si, and on a conventional Spherisorb S5W stainless steel column (10) is illustrated in Fig. 4.

Retention on non-end capped bonded stationary phase materials such as octadecylsilyl (ODS) again appears to be mediated largely by surface silanols (8). Differences in elution sequence, but generally poorer peak shapes, were obtained for some amiodarone analogues on Spherisorb S5 ODS1 as compared to unmodified silica (Fig. 2) and this difference in elution sequence did prove useful in the definitive identification of desethylamiodarone (13). In contrast, a Spherisorb S5P phenyl-modified material has proved useful in the analysis of the analgesic meptazinol since a better peak shape was obtained on this material than on several unmodified silica columns at a range of eluent pHs (16). However, no further indications for the use of such materials have been found.

One restriction in the use of non-aqueous ionic eluent/silica column systems is that certain compounds are poorly retained even when using



4. Comparison of retention (k' values) of 32 basic drugs. Eluent: methanolic ammonium perchlorate (10 mM) + 1 ml/l methanolic NaOH (0.1 M), apparent pH 6.7. Detection: UV, 254-260 nm. Injection: 20-100 ul of methanolic solutions (ca. 10 mg/l) of each analyte. Columns: 'ChromSpher Si' and 'Spherisorb Si' ChromSep® cartridges (200 x 3 mm i.d., flow-rate: 0.7 ml/min, cf. ref. 15) and 'Spherisorb S5W' (125 x 5 mm i.d. stainless steel column, flow-rate: 2 ml/min, cf. ref. 10).

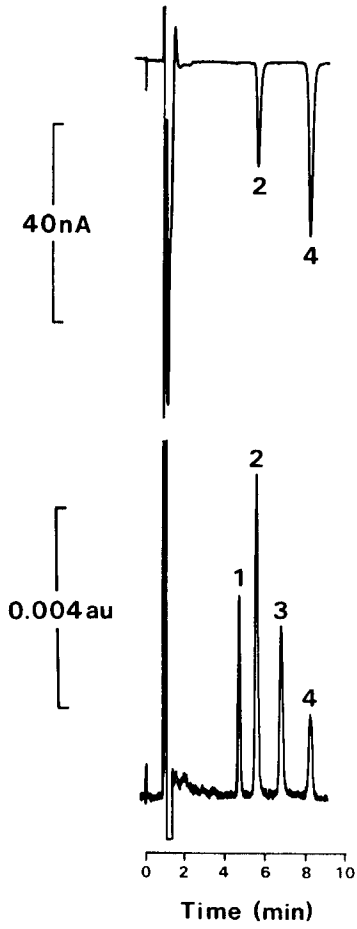
low ionic strength eluents. The recent introduction of micro-particulate strong cation-exchangers suggested an additional approach and two of these materials, Zorbax 300 SCX (Du Pont) and Spherisorb S5 SCX (Phase Separations), have been evaluated. The analytes studied were well retained on both materials, a high proportion of acetonitrile and a relatively high ionic strength being required to promote elution in a reasonable time, and good peak shapes were obtained (Figs. 5 & 6).

2. Modes of Detection

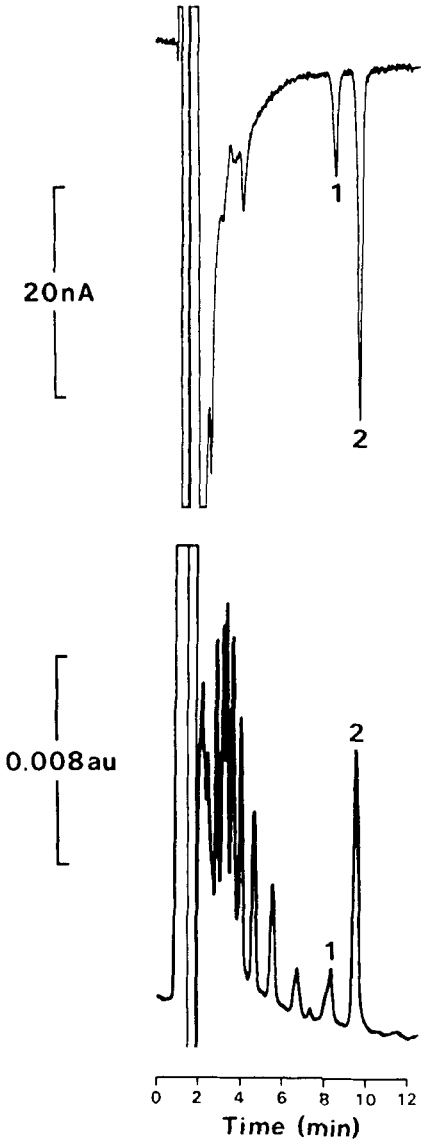
2.1. UV and Fluorescence Detection

The use of HPLC grade methanol or acetonitrile and perchloric acid/ ammonium perchlorate gives an eluent with a low UV cut-off (ca. 200 nm). This can be advantageous especially when compared to conventional adsorption chromatography systems employing chlorinated solvents (Fig. 7). However, in our experience the use of relatively non-selective wavelengths (200-220 nm) can pose problems if high-sensitivity work on biological extracts is contemplated. It is noteworthy that many reports advocating such wavelengths for particular analyses make no mention of potential sources of interference.

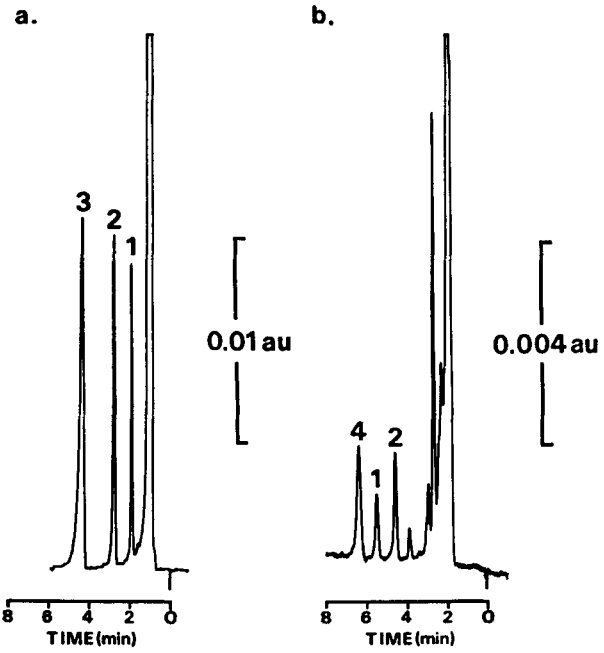
Many beta-blockers and some other cardioactive drugs of interest will exhibit fluorescence thus giving a selective and sensitive mode of detection. We have normally operated Kratos-Schoeffel FS 970 fluorescence detectors without an emission filter when low excitation



5. Separation of propafenone & 5-hydroxypropafenone using a strong cation-exchanger. Column: 125 x 5 mm i.d. Spherisorb S5 SCX. Eluent: acetonitrile:methanol (4+1) containing ammonium perchlorate (15 mM, apparent pH 4.5). Detection: UV, 215 nm & electrochemical, + 0.9 V vs. Ag/AgCl. Flow-rate: 2 ml/min. Injection: 100 ul methanolic solution. Peaks: 1 = propafenone, 2 = 5-hydroxypropafenone, 3 = N-depropyl-propafenone degradation product (all 0.5 mg/l), 4 = prenalterol (internal standard) (0.2 mg/l).



6. Analysis of lignocaine in Subtilisin A placental digests. Column: 250 x 5 mm i.d. Spherisorb S5 SCX. Injection: 100 ul of methyl tert.-butyl ether extract (pH 11) of digest (100 ul). Eluent: acetonitrile: methanol (4+1) containing ammonium perchlorate (15 mM) adjusted to apparent pH 5.7 using methanolic NaOH (0.1 M). Flow-rate: 2 ml/min. Detection: UV, 220 nm and electrochemical, + 1.0 V vs. Ag/AgCl. Peaks: 1 = lignocaine (original tissue concentration 1.6 mg/kg wet wt.), 2 = bupivacaine (internal standard).



7. Comparison of HPLC amiodarone methods. Sample: plasma from patient treated with 200 mg/day amiodarone. (a) Column: 125 x 5 mm i.d. Spherisorb S5W silica. Injection: 100 ul of methyl tert.-butyl ether extract (pH 4.5) of plasma (200 ul). Eluent: methanolic ammonium perchlorate (10 mM) + 1 ml/l methanolic NaOH, apparent pH 6.7. Flow-rate: 2 ml/min. Detection: UV, 240 nm. (b) Method of Lesko et al. (17) except extraction performed at pH 4.5 not 3.8. Column: 250 x 4.6 mm i.d. Spherisorb S5W silica. Injection: 100 ul of extract of plasma (500 ul) in 150 ul chloroform. Eluent: chloroform:methanol:ammonium hydroxide (98.95+1.0+0.05). Flow-rate: 0.8 ml/min. Detection: UV, 280 nm (not possible to back-off zero at 254 nm). Peaks (plasma conc in brackets): 1 = desethylamiodarone (0.7 mg/l), 2 = amiodarone (0.9 mg/l), 3 = fenethazine & 4 = L8040 (2-ethyl-3-(3,5-dibromo-4-gamma-di-n-propylaminopropoxybenzoyl)benzothiophene) (internal standards).

wavelengths (200-220 nm) are used. This gives an increased signal-to-noise ratio with many analytes as opposed to the use of higher excitation wavelengths together with the lowest (370 nm) filter supplied with the instrument. However, the use of lower cut-off filters (270 and 320 nm) (McPherson, available in the U.K. from Intersci, Cambridge) may give an increased signal-to-noise ratio for certain analytes. Finally, fluorescence detection following post-column reaction with o-phthaldialdehyde/2-mercaptoethanol may be used for primary aliphatic amines although certain compounds such as tocainide did not react under the conditions used (10), possibly for steric reasons.

2.2. Electrochemical Oxidation Detection

A feature of non-aqueous ionic eluent systems is that higher applied potentials may be used than with conventional aqueous mobile phases while maintaining good sensitivity. Most retained analytes except primary aliphatic amines, quaternary ammonium compounds and some others such as benzodiazepines will respond, the magnitude of the response being influenced primarily by the electroactive moieties present and the applied potential (Table 1). The eluent pH is also important, an apparent pH of 6.7 being a compromise between retention, peak shape and response especially of secondary and tertiary aliphatic amines (Section 1.1). The nature and activity of the electrode are also important and detailed comments on electrochemical oxidation detection in basic drug analyses are available (10).

It should be emphasised that the electrochemical detector is in effect a 'reaction detector' and requires more thought in routine use than

TABLE 1. Optimal Electrochemical Detection Conditions for Certain Functional Groups

(Column: 125 x 5 mm i.d. Spherisorb S5W silica. Eluent: methanolic ammonium perchlorate (10 mM) + 1 ml/l methanolic NaOH (0.1 M), apparent pH 6.7. Flow-rate: 2 ml/min. Working electrode: V25 grade glassy carbon (Ref. 9). Reference electrode: Ag/AgCl.

Functional group	Optimum oxidation voltage (V)	Approximate detection limit (ng)	Typical standing current (nA)
Phenol, aromatic amine	0.7	0.1	2-5
Phenothiazine sulphur	0.8	0.1	5-10
Imidazoyl nitrogen, indole	0.9	0.2	10-20
Tertiary aliphatic amine	1.0	0.5	50-100
Secondary aliphatic amine	1.2	2	200-500
Primary aliphatic amine	1.6	20	>2000
Pyridyl nitrogen, quaternary ammonium compound	>1.6	-	-

conventional UV or fluorescence detectors. On the other hand, running costs are minimal and enhanced sensitivity may be conferred for analytes such as 5-hydroxypropafenone and lignocaine which possess relatively poor UV but good electrochemical detection characteristics (Figs. 5 & 6). We use a V25 grade glassy carbon working electrode (Le Carbone) in a stainless steel wall-jet assembly (a version of which is available commercially from Thames Chromatography, Maidenhead, UK, Model TC100). Saturated methanolic potassium chloride is used as the electrolyte in the reference electrode to minimise the risk of air bubble formation within the electrode. (N.B. It is important to treat

the silver wire in the reference electrode assembly with methanolic or aqueous silver nitrate solution to ensure the presence of a film of silver chloride before use). Other glassy carbon electrode assemblies may be used although the response characteristics may vary (10). It is not advisable to use carbon-paste electrodes, however.

One problem in routine operation is that deactivation of the response may occur. This is especially noticeable with secondary aliphatic amines, the more so if extracts of specimens obtained post-mortem have been analysed. Re-polishing of the electrode using a felt pad and an aqueous slurry of 2u alumnia is required to fully restore the response (10). The standing current provides a useful indicator during routine operation and the values which should be attainable at different applied potentials are given in Table 1. Loss of response is normally accompanied by a decreased current while excessive noise due to, for example, a contaminated eluent is usually paralleled by an increase in the standing current. Operation for any length of time with a seriously contaminated eluent will also necessitate re- polishing since the excessive current will tend to 'etch' the electrode surface. Prolonged eluent re-circulation with, for example, injection of methyl tert.-butyl ether sample extracts may also give rise to a loss of response similar to that observed with electrode deactivation but here replacement of the eluent may restore the response.

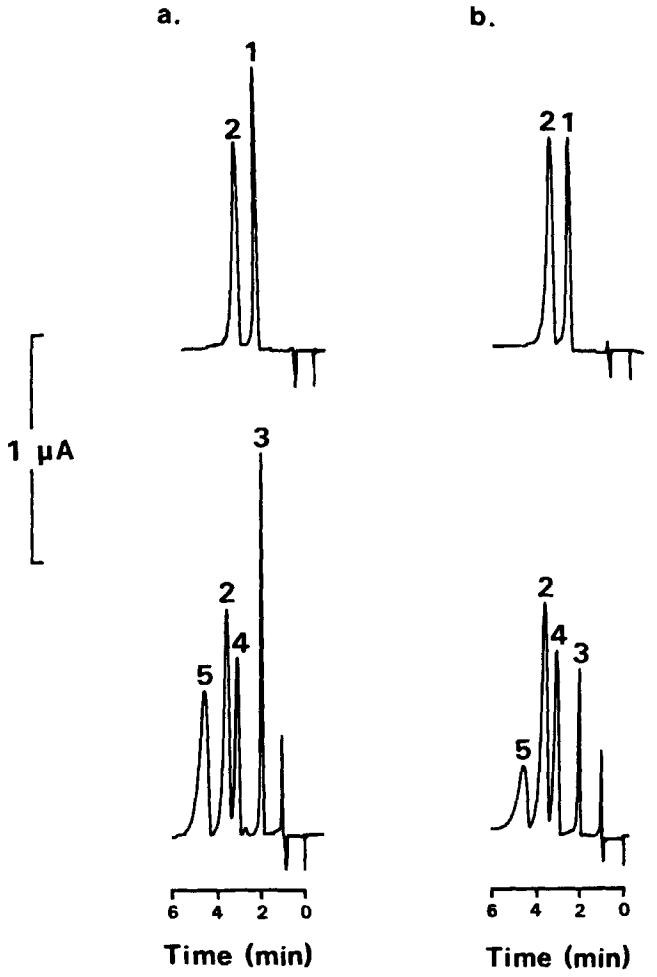
Finally, the nature of the ionic modifier may influence the response of certain functional groups. Thus, at constant pH and ionic strength, the use of ammonium acetate rather than ammonium perchlorate results in a decreased response for nortriptyline (secondary aliphatic amine)

and the phenothiazine methdilazine at + 1.2 V, the standing current and the response of the remaining compounds studied being largely unchanged (Fig. 8). Whether other modifiers would give rise to more selective/sensitive responses for particular analytes is a topic for further study.

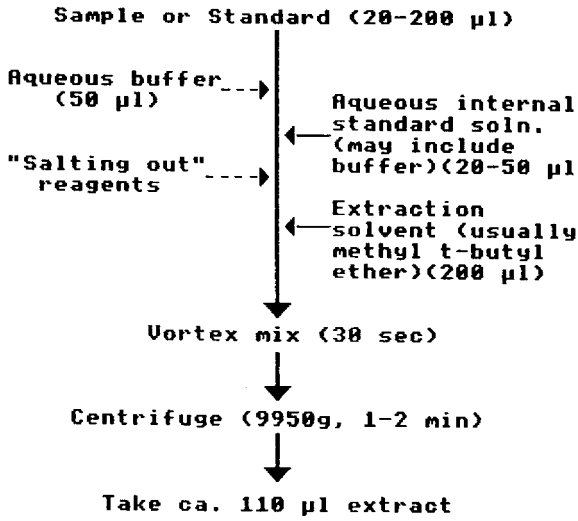
3. Sample Preparation and System Operation

As noted above, a major reason for the development of the assay system described was the need to analyse solvent extracts directly and a flow-diagram of a generalised micro-extraction procedure as developed in our laboratory is given in Fig. 9. We use glass extraction vessels (60 x 5 mm i.d., Dreyer tubes) in order to simplify extract removal and thus to minimise the risk of contamination from the aqueous phase. The Eppendorf 5412 high-speed centrifuge gives rapid phase separation and minimises the risk of emulsion formation. Hamilton gas-tight luer-fitting glass syringes fitted with Hamilton repeating dispensers and stainless steel needles are used for solvent and reagent additions whenever possible. Methyl tert.-butyl ether is often used as the extraction solvent and, at an appropriate pH, gives good extraction efficiencies with most of the analytes studied. This compound has a relatively low UV cut-off as compared to other solvents such as n-butyl acetate while other ethers such as di-isopropyl require stabilisation with compounds such as hydroquinone.

This approach is low-cost, suitable for one-off clinical analyses and extract injection can be automated - we use 0.5 ml (30 x 8 mm i.d.)



8. Influence of ionic modifier on electrochemical response. Column: 125 x 5 mm i.d. Spherisorb S5 Phenyl. Injection: 20 ul of methanolic solutions (10 mg/l). Flow-rate: 2 ml/min. Detection: electrochemical, + 1.2 V vs. Ag/AgCl. Eluent: (a) methanolic ammonium perchlorate (10 mM) + 1 ml/l methanolic NaOH (0.1 M), apparent pH 6.7, (b) methanolic ammonium acetate (10 mM) + 6 ml/l 10% (v/v) glacial acetic acid in methanol, apparent pH 6.7. Peaks: 1 = meptazinol, 2 = imipramine, 3 = nortriptyline, 4 = amitriptyline, 5 = methdilazine.



9. Generalised flow-diagram of solvent micro-extraction procedures.

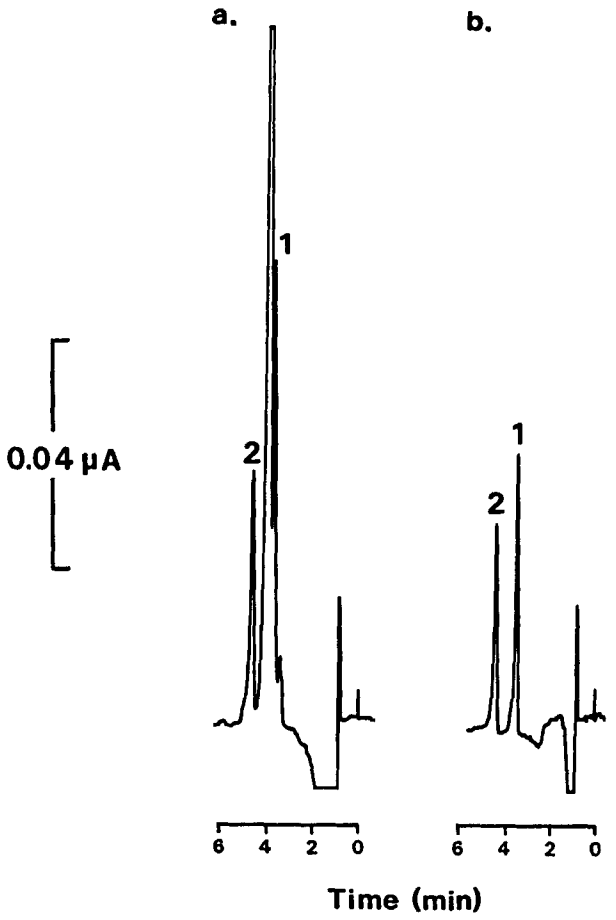
capped disposable polypropylene tubes (Sarstedt) and SV-S6 PTFE adaptors (Chromacol) as sample holders in the Perkin-Elmer ISS-100 auto-sampler to reduce costs. The extracts obtained are generally free from endogenous sources of interference and relatively large volume sample injections may be performed in a "non-eluting" (zero ionic strength) solvent with no loss of efficiency (8). This property is advantageous when used with the AASP® automated sample preparation system (Varian), enabling methanol to be used to elute the analyte(s) from the extraction cartridge in a sharp band which may then be analysed directly (C K Lim, Personal Communication). The assay of the relatively water-soluble drug atenolol in breast milk provides an example of a more complex procedure whereby a pre-extraction into methyl tert.-butyl ether can be used to remove a lipophilic

contaminant. Subsequently, the extraction of atenolol may be accomplished by addition of NaCl (50 mg), 10 M NaOH (50 ul) and 10% heptafluorobutanol in methyl tert.-butyl ether (Fig. 10). Thus, "salting-out" of relatively water-soluble analytes can prove useful as in the above case although emulsion formation may ensue if salts are added to excess.

The expected life of the analytical columns varies from months to years although cleaning and slurry re-packing the tops of heavily-used columns can give prolonged life. The use of stainless steel 2u mesh to retain the column packing is to be preferred since in our experience the 2u porous frits advocated by many manufacturers are prone to blockage and need constant attention. We use conventional male end-fittings with 'ferrules' made from ca. 1/8" lengths of 1/8" o.d. PTFE tubing even at the inlet end of the column and this minimises the risk of physical damage to the stainless-steel fittings and tubing from over-tightening. As noted above, eluent re-circulation may be employed to minimise running costs although care must be taken to ensure that neither the resolution of the system nor any electrochemical response(s) are adversely affected.

CONCLUSIONS

The approach to the HPLC analysis of basic drugs described here has developed in our laboratory over the last 8 years and has formed the basis of our cardioactive drug assay service. We find silica column/non-aqueous ionic eluent systems to be relatively predictable and easy to use as compared to conventional bonded-phase/aqueous methanol or



10. The analysis of atenolol in breast milk. Column: 125 x 5 mm i.d. Spherisorb S5W silica. Eluent: methanol containing d-10-camphor-sulphonic acid (1 mM). Flow-rate: 2 ml/min. Detection: fluorescence, excitation 200 nm, no emission filter (Kratos-Schoeffel FS 970). (a) Injection: 100 ul of 10 % (v/v) heptafluorobutanol/methyl tert.-butyl ether extract (pH 14) of breast milk (250 ul) (see text). (b) As (a) but after pre-extraction with methyl tert.-butyl ether (pH ca. 7) alone. Peaks: 1 = atenolol (breast milk concentration 0.63 mg/l), 2 = benzimidazole (internal standard).

acetonitrile eluent systems. However, there is continual pressure for enhanced separating power and more sensitive/selective detection. Porous graphite flow-through electrochemical cells offer the possibility of coulometric detection and such cells are now available in a dual-electrode configuration (Coulochem). Although relatively expensive, such instruments may give an enhanced signal-to-noise ratio for certain analytes and are more flexibile in routine use than wall-jet assemblies. Post-column derivatisation prior to fluorescence detection is another area which should be further explored.

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REFERENCES

1. Holt, D. W., Therapeutic drug monitoring of antiarrhythmic drugs. In 'Contempory Issues in Clinical Biochemistry', Widdop, B., Ed., Churchill Livingstone, Oxford, 1985, p. 154.

2. Lagerstrom, P.-O. and Persson, B.-A., Liquid chromatography in the monitoring of plasma levels of antiarrhythmic drugs, J. Chromatogr. 149, 331, 1978.

3. Kabra, P. M., Chen, S.-H. and Morton, L. J., Liquid-chromatographic determination of antidysrhythmic drugs: procainamide, lidocaine,

quinidine, disopyramide and propranolol, Ther. Drug Monit. 3, 91, 1981.

4. Wesley, J. F. and Lasky, F. D., High performance liquid chromatographic analysis of the antiarrhythmic drugs procainamide, disopyramide, quinidine, propranolol and metabolites from serum extracts, Clin. Biochem. 14, 113, 1981.

5. Ramsey, J. and Campbell, D. B., An ultra rapid method for the extraction of drugs from biological fluids, J. Chromatogr. 63, 303, 1971.

6. Flanagan, R. J. and Withers, G., A rapid micro-method for the screening and measurement of barbiturates and related compounds in plasma by gas-liquid chromatography, J. Clin. Pathol. 25, 899, 1972.

7. Flanagan, R. J., Storey, G. C. A. and Holt, D. W., Rapid high-performance liquid chromatographic method for the measurement of amiodarone in blood plasma or serum at the concentrations attained during therapy, J. Chromatogr. 187, 391, 1980.

8. Flanagan, R. J., Storey, G. C. A., Bhamra, R. K. and Jane, I., High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. J. Chromatogr. 247, 15, 1982.

9. Flanagan, R. J. and Jane, I., High-performance liquid chromatographic analysis of basic drugs on silica columns using

non-aqueous ionic eluents. I. Factors influencing retention, peak shape and detector response, J. Chromatogr. 323, 173, 1985.

10. Jane, I., McKinnon, A. and Flanagan, R. J., 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, J. Chromatogr. 323, 191, 1985.

11. Cole, S. J. C., Flanagan, R. J., Johnston, A. and Holt, D. W., Rapid high-performance liquid chromatographic method for the measurement of verapamil and norverapamil in blood plasma or serum, J. Chromatogr. 218, 621, 1981.

12. Storey, G. C. A., Holt, D. W., Holt, P. and Curry, P. V. L., High-performance liquid chromatographic measurement of amiodarone and its desethyl metabolite: methodology and preliminary observations, Ther. Drug Monit. 4, 385, 1982.

13. Flanagan, R. J., Storey, G. C. A., Holt, D. W. and Farmer, P. B., Identification and measurement of desethylamiodarone in blood plasma specimens from amiodarone-treated patients, J. Pharm. Pharmacol. 34, 638, 1982.

14. Bhamra, R. K., Flanagan, R. J. and Holt, D.W., Measurement of penbutolol and 4-hydroxypenbutolol in plasma or serum by HPLC, Biomed. Chromatogr. 1, 140, 1986.

15. Walker, S., Flanagan, R. J. and Holt, D. W., HPLC measurement of penticainide and desalkylpenticainide in biological fluids, Biomed. Chromatogr. 2, 34, 1987.

16. Flanagan, R. J. and Ward, N., HPLC measurement of meptazinol in plasma using electrochemical oxidation detection, Biomed. Chromatogr. 1, 180, 1986.

17. Lesko, L. J., Marion, A., Canada, A.T. and Haffajee, C., High-pressure liquid chromatography of amiodarone in biological fluids, J. Pharm. Sci. 70, 1366, 1981.