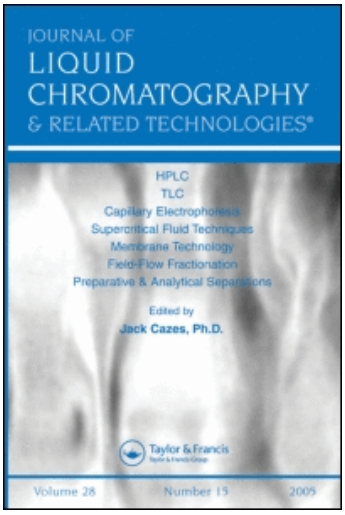

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Practical Aspects of LC/EC Determinations of Pharmaceuticals in Biological Media

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PRACTICAL ASPECTS OF LC/EC DETERMINATIONS OF PHARMACEUTICALS IN BIOLOGICAL MEDIA

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ABSTRACT

Liquid chromatography with electrochemical detection (LC/EC) has proven itself to be a very useful technique for the determination of electrochemically oxidizable or reducible compounds in complex matrices. Practical aspects of the application of LC/EC to pharmaceuticals in biological samples are discussed. These have been gleaned from several years experience with the determination of over a dozen compounds. The aspects discussed are a comparison of EC and UV detection which facilitates a choice between the two, and common problems including electrode coating, late eluters, detector temperature dependence and baseline instability. Various possible solutions to these problems are considered.

INTRODUCTION

Since the invention of electrochemical detectors (EC) for liquid chromatography (LC) just over a decade ago, their use has grown exponentially, so that presently there are over one thousand literature reports of their use. For the liquid chromatographic determination of small, readily oxidized molecules, electrochemical detection is clearly established as being superior to the more generally

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applicable and available ultraviolet (UV) detectors. For more than four years we have used EC detectors in the LC determination of a variety of pharmaceuticals in biological samples. These compounds have generally not been as ideally suited to EC detection as the catecholamines and related compounds for which EC was developed and for which it has been most frequently used. Nevertheless, LC/EC has proven to be a very useful technique. Several practical aspects of our experience with LC/EC are presented here.

MATERIALS AND METHODS

Reagents

Enviroxime, zinviroxime, hexestrol, enviradene and its cis-olefin isomer, frenazole, diethylstilbestrol, cis-diethyl-stilbestrol, and pergolide, were synthesized at the Lilly Research Laboratories. N-(4-hydroxyphenyl)-propionamide and N-(4-hydroxy-3-methylphenyl) acetamide were a gift from P.T.Kissinger, Purdue University. All other compounds were obtained commercially.

Distilled deionized water was used for preparation of mobile phases. Distilled-in-glass benzene, methanol and acetonitrile were obtained from Burdick and Jackson (Muskegon, MI), HPLC grade methanol from Tedia (Fairfield, OH) and analytical grade methanol from MCB Manufacturing Chemists (Cincinnati, OH). All other reagents were analytical reagent grade.

Equipment

The HPLC systems consisted of a solvent delivery pump and autoinjector (Models 6000A and WISP 710B, Waters Associates, Milford, MA), a guard column packed with pellicular packing material (CO:PELL ODS, Whatman, Clifton, NJ), a 250 x 4.6 mm reverse phase column, a column temperature controller and an electrochemical detector (Models LC-22/23

and LC-4 with a TL-5 or TL-5A glassy carbon detector cell, Bioanalytical Systems, W. Lafayette, IN). Detector potential was referenced to a Ag/AgCl/3 M NaCl reference electrode. A UV detector (Model 450, Waters Associates) was either substituted for the electrochemical detector or connected between the column and the electrochemical detector. The fluorescence detector used was a dual monochromator instrument (Model 650-10 S, Perkin Elmer, Norwalk, CT). Detector response was monitored by a chart recorder and a central chromatographic data acquisition computer system.

Column-switching was done as described previously (1), except that a silica column (Pre-SAT, Applied Science, State College, PA) was placed between the auxiliary pump and the 3cm reverse phase column, and a 250 x 4.6mm column was placed downstream from the 3 cm column, to provide a constant backpressure.

For the investigation of the temperature dependence of the electrochemical detector, the EC cell was thermostatted independently of the chromatographic column, as described previously (2).

Chromatography

Enviroxime and zinviroxime were chromatographed on an octylsilane column (Zorbax C8, Dupont Instruments, Wilmington, DE) with a mobile phase of 0.14 M sodium acetate and methanol (35:65), containing 3 mg of disodium EDTA per liter. The flow rate was 0.9 ml/min and the column temperature 28°C. The stilbestrols were chromatographed on an octadecylsilane column (Zorbax C18) with the same mobile phase as above. The flow rate was 1.5 ml/min and column temperature 30°C. Enviradene was also chromatographed on the Zorbax C18 column, but with a mobile phase of 0.2 M sodium acetate and methanol (25:75), containing 3mg EDTA/liter. The flow rate was 1.3 ml/min and column temperature was 28°C. For pergolide a nitrile column (Zorbax CN) was used, with a mobile phase of acetonitrile/methanol/0.1 M ammonium acetate (30:25:50). The flow rate was 2 ml/min. Acetaminophen and its analogs were chromatographed on an octadecyl column (Zorbax C8) with 0.1M citrate (pH 4.0) and methanol

(86:14) as the mobile phase. The flow rate was 1 ml/min and the column temperature was 28°C. The catecholamines were chromatographed with 1.4% monochloroacetate (pH 3.0) and methanol (95:5) as mobile phase. Sodium octyl sulfonate (25mg/l) and disodium EDTA (38mg/l) were also added to this solution. These amines also were run on a Zorbax C8 column at 28°C and a flow rate of 1 ml/min. The octadecylenol compound was assayed on a C18 column (Zorbax) with a mobile phase of 55% n-propanol. All mobile phases were filtered through a 0.2 μm pore filter (Rainin Inst. Co., Woburn, MA) and sonicated under vacuum prior to use.

RESULTS AND DISCUSSION

EC vs. UV Detection

The choice of an LC detector for an assay under development can involve a number of factors. Chief among these are signal-to-noise ratio (or detection limit), specificity and convenience. UV detection, which is the most universally used method, is the common alternative to EC detection. The relative merits of the two detectors, particularly in regard to the three factors listed above, were gleaned from consideration of assays developed for a number of compounds, including 1) two stilbestrols, 2) two pairs of antirhinovirals, 3) an octadecylenol, and 4) an ergot derivative. These assays are first discussed individually.

Diethylstilbestrol (DES) is a synthetic estrogen capable of producing all the pharmacologic and therapeutic responses attributed to natural estrogens. An assay was developed for DES (by definition the trans isomer) and its corresponding cis isomer involving a benzene extraction of the compound and an internal standard (benzestrol) from either plasma or urine. The plasma or urine was treated with a commercial glucuronidase/sulfatase preparation from H. pomatia prior to extraction if total drugs (free and conjugated) were to be determined. The organic extracts were evaporated and the residues reconstituted and injected. Detection parameters were chosen based upon electrochemical and spectral scans. Hydrodynamic voltammograms obtained for the three compounds are displayed in Figure 1. With two conjugated aromatic

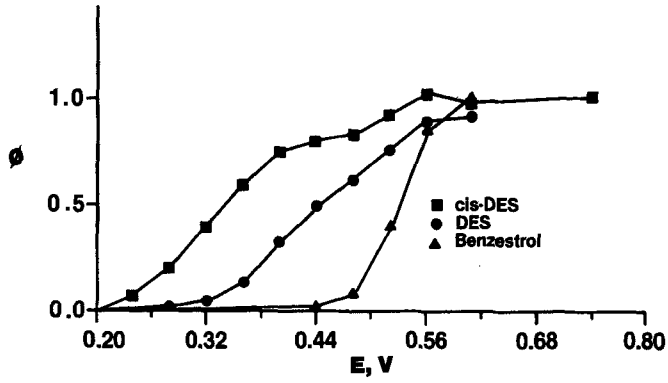
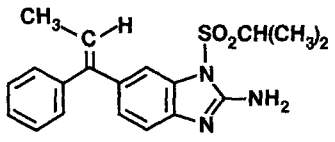


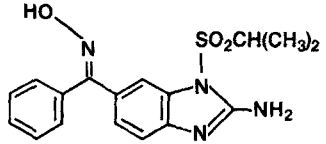
FIGURE 1. Hydrodynamic voltammograms of stilbestrols under the LC conditions described in the materials and methods section.

hydroxy groups, the stilbestrols are readily oxidized. Hydrodynamic $E_{\frac{1}{2}}$'s determined from the data in Figure 1 were +0.4 and 0.3 V for DES and cis-DES respectively. A detector potential of +0.70 V was used, although a somewhat lower potential would have sufficed to provide diffusion-limited oxidation. The ultraviolet absorption of DES and cis-DES was found to be moderate (ε_{262}=8635 for both isomers). Typical chromatograms of a plasma extract comparing UV and EC detection are depicted in Figure 2. EC detection can be seen to be clearly preferred in terms of freedom from interferences and signal-to-noise ratio (S/N). The detection limits realized were approximately 1 ng/ml of plasma for EC and 10 ng/ml for UV. Some increase in UV signal could be realized by operation at 210nm, but a concomitant increase in background noise would result in only a small increase in overall S/N.

Enviradene, whose structure is shown below, is the trans-methyl olefin analog of the antirhinoviral oxime enviroxime, whose determination in plasma has been previously reported (1). Zinviroxime is the cis-oxime



Enviradene



Enviroxime

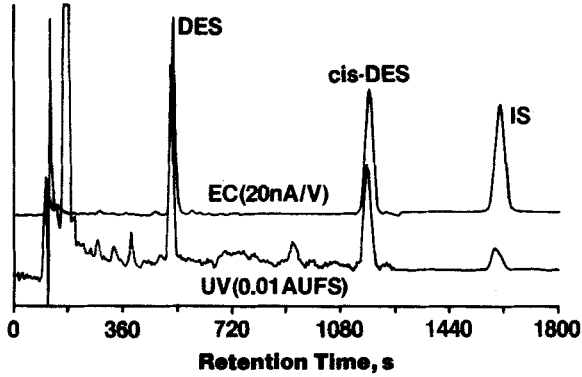


FIGURE 2. Serial UV and EC chromatograms of a 100ng per ml plasma extract.

isomer of enviroxime. The difference in the structures of these molecules is solely at the exocyclic double bond position. Plasma levels of enviradene may be determined by a procedure very similar to that reported for enviroxime, with the mobile phase altered slightly and the electrode potential increased to +0.90 V. The oxidations of enviroxime and zinviroxime were reasonably similar (hydrodynamic $E_{\frac{1}{2}}$=+0.72 and +0.81 V, respectively) and EC was clearly preferred over UV detection for plasma assays. Interestingly, and in contrast to the oximes, enviradene and its isomeric cis-olefin are more difficult to oxidize (Figure 3), with an $E_{\frac{1}{2}}$ of +0.8 and +0.94 V, respectively. Also, their oxidation potentials show a greater difference between each other. In addition, the methyl olefins have higher molar absorptivities (ε_{268}=17,800) than those of enviroxime and zinviroxime (ε_{272}=14,200). As as result, while EC S/N ratios are marginally better than UV for enviradene, S/N is better using UV detection for the cis-olefin. Figure 4 shows a plasma extract chromatographed on a system with EC and UV detectors set up in series. No significant interferences were observed in chromatograms with either detector. However, the use of UV detection was noted to have several advantages. The wavelength chosen was one at which the two isomers have identical molar absorptivities. Thus if peak areas are calculated, the two isomers can be quantitated from a single standard curve. The linearity of the UV detector extended to greater than 10μg on column,

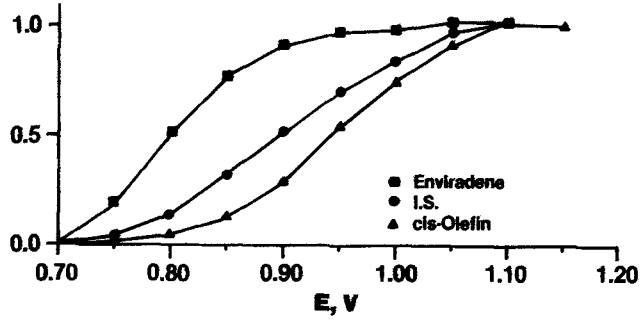


FIGURE 3. Hydrodynamic voltammograms of the methyl olefin antivirals under the LC conditions described in the materials and methods section.

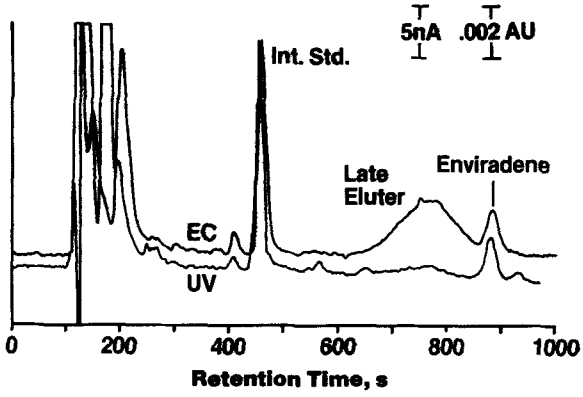


FIGURE 4. EC and UV chromatograms of an extract of a plasma sample spiked with 50 ng enviradene per milliliter

while the EC was limited to 400 ng. Also, the late eluters encountered with the EC detector (see Figure 4) were relatively much smaller on UV.

A third type of compound for which EC and UV detection were compared was an octadecylenol compound, CH_3-$(CH_2)_{17}$-OR. A hydrodynamic voltammogram for this compound is depicted in Figure 5. The oxidation of this molecule involves the enol oxygen, so that octadecylalcohol is probably liberated in the process. Although the half-wave potential for this oxidation is relatively low, the wave is far from reversible. The

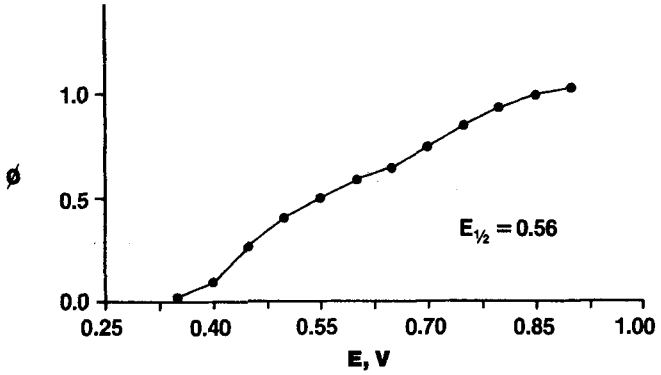
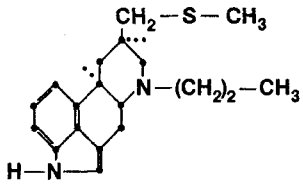


FIGURE 5. Hydrodynamic voltammogram of an octadecylenol compound obtained using the chromatographic conditions described in the materials and methods section.

UV absorption spectrum of this compound exhibited a maximum at 245 nm with an molar absorptivity of 9720. A procedure was developed for the determination of this compound in plasma at the 1 μg/ml level. The octadecylenol was isolated from plasma by precipitation of proteins with the addition of 1 volume of n-propanol, followed by 1 volume acetonitrile. This procedure gave chromatograms without EC or UV interferences. The minimum quantity which could be detected by EC (at a potential of +0.85 V) was 5 times lower than that by UV. However, a large EC response from non-retained components, severe coating problems (discussed further below) and the low solubility of salts in the mobile phase forced the abandonment of EC detection. Thus, UV detection was employed for this determination.

The fourth assay to be considered here is one developed for pergolide, the structure of which is shown below. Pergolide is an ergot



Pergolide

alkaloid derivative currently under study for the treatment of Parkinson's disease. It contains a single substituted aromatic amine group and is not easily oxidized (hydrodynamic $E_{\frac{1}{2}}$~+0.86 V at pH 7). Its molar absorptivity is relatively low (ε_{280}=5300) also. A procedure was developed for the determination of pergolide in feed samples in support of animal toxicology studies. The feed samples were dissolved in 10 volumes of 80:20 methanol/0.1M HCl. This solution was passed over alumina and an aliquot injected. The absolute detection limit using UV detection was 20ng on column, which was insufficient to allow detection of pergolide at the desired levels. The use of EC lowered the detection limit to 2ng on column, but a large t_o response was observed (see Figure 6) as were many large late eluters. Instead of either EC or UV detection, fluorescence detection was used, since it provided detection limits superior to EC and lacked interferences (Figure 7).

Several general observations relative to the choice between EC and UV detection for LC determination of drugs in biological matices may be drawn from the assays just discussed. First of all, the relative S/N of the two detectors is frequently an important consideration. For UV detection, the signal (peak area or height) obtained for different analytes (assuming comparable retention times) is proportional to their extinction coefficient at an accessible ultraviolet or visible wavelength. Signal to noise with EC detection is more complex. The

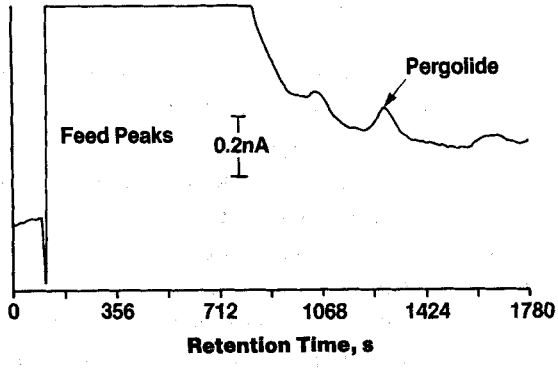


FIGURE 6. LC/EC chromatogram of an extract of feed spiked with 2 ppm of pergolide.

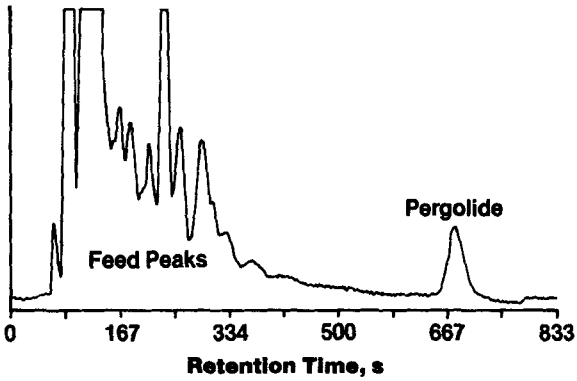


FIGURE 7. LC/Fluorescence chromatogram of an extract of feed that was spiked with 2 ppm of pergolide. The excitation wavelength was 285 nm (slit 10nm) and the emission wavelength was 345 nm (slit 5nm).

signal for conventional amperometric detectors is a function of their configuration, the working electrode potential of the detector, and the diffusion coefficient, half-wave potential and reversiblity of the analyte under the chromatographic conditions. The noise of a conventional solid electrode detector is a function of many factors, but it is generally relatively constant from 0.0 volts out to the potential at which solvent oxidation (or reduction) begins to occur. From there on, background current and noise increase exponentially. Thus as the half-wave potential of a theoretical analyte approaches the oxidation potential of the the solvent, the maximum realizable S/N decreases.

For readily oxidized molecules, such as catecholamines or the stilbestrols discussed above, the S/N of EC detection is clearly better than that of UV detection. As analytes of increasing oxidation potential are investigated, S/N will decrease for EC detection. As analytes with larger extinction coefficients are investigated, S/N for UV detection will increase. Thus with increasing $E_{\frac{1}{2}}$ or ε or both, a point will be reached at which S/N for the two detectors is equivalent. Analytes with even higher $E_{\frac{1}{2}}$ or ε will exhibit a better S/N by UV detection. The results of the four assays just described confirm this hypothesis. Figure 8 includes a point for each compound, and the line represents the points at which S/N is equal for EC and UV. The regions in which EC or

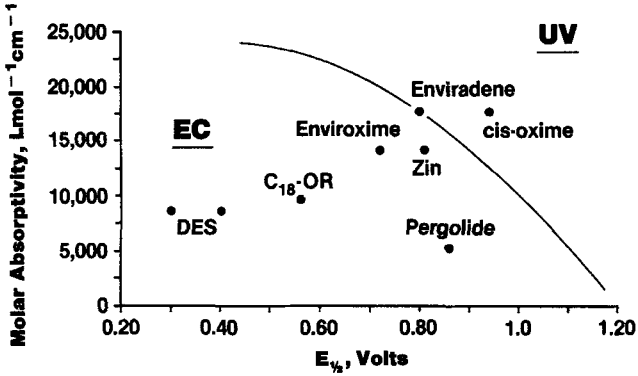


FIGURE 8. EC versus UV detection for LC-Dependence on molecular parameters. The compounds to the left of the line have lower absolute detection limits by EC detection than by UV detection. The opposite is true for compounds to the right of the line.

UV detection are preferred are clear. Data for additional analytes, particularly some with high $E_{\frac{1}{2}}$/low ε and high ε/low $E_{\frac{1}{2}}$, will allow a better definition of the location of this line. Nonetheless, it is apparent that a guideline can be established for making a choice between EC and UV detection when signal-to-noise ratio is an important consideration.

A number of additional factors influence the choice of detector for a given determination. The advantages of EC and UV detection relative to each other are summarized in Table 1. Many of the factors listed there were encountered in assays described above. The particular advantages of the EC detector are its sensitivity and specificity. For electrochemically active analytes, EC detection has a superior absolute sensitivity to UV. For an electrochemically active compound, the specificity of EC detection can make assay development much easier. UV detection on the other hand is useful for more analytes. UV detection is more convenient, since the condition of electrodes is not a consideration, as is the case with EC detection. An isobestic point may be chosen so that more than one compound can be quantitated from a single standard curve. For an internal standard to be maximally effective, its chemical properties should closely approximate those of the analyte. In

TABLE 1

	EC	UV
Advantages	Sensitivity	Ease of Use
	Specificity	Universality
		Isobestic Points
		Choice of Int. Stds.
Disadvantages	Coating	Drug Interferences
	Ref. Electrode Stability	Temperature Sensitivity
	Temperature Sensitivity	
	Late Eluters	

this regard UV detection has an advantage since internal standards are more readily found which approximate the UV properties of an analyte, than those which have similar electrochemical properties.

The disadvantages of the two detectors relative to each other are also summarized in Table 1. Some of the potential problems with EC detection, including electrode coating, temperature sensitivity and late eluters are discussed in detail below. UV detectors are subject to fluctuations in response with room temperature also. The more universal nature of UV detection also means that more of the components of the sample matrix will be detected and so are potential interferences. Thus background can be more of a problem with UV detection than with EC detection.

Common Problems-Electrode Coating

Electrode inactivation, or coating, is common in conventional voltammetric work. The occurrence of coating in LC/EC has been previously noted (3,4), but it is a much less frequent problem. This is a function both of the relatively low amounts of compounds chromatographed (picograms to a few micrograms) and the relatively high velocity of the mobile phase over the working electrode surface. In our work we have encountered a number of examples of compounds which exhibit

coating problems, however. The details of these problems, as well as a series of possible solutions which were investigated, are discussed below.

When injecting samples containing high concentrations of enviroxime and zinviroxime (0.4 to 1.5 μg/ml) it was noted that the peak height of the internal standard hexestrol decreased considerably during the chromatography of a single set of samples. Further investigation revealed that the sensitivity of the detector to enviroxime and zinviroxime decreased also. A series of experiments were performed to determine the source of the problem. Various combinations of the three compounds, all at 1 μg per injection, were repeatedly injected. Between experiments the glassy carbon working electrode was repolished. These experiments are summarized in Table 2. The compound(s) injected are listed on the left and % decreases in peak height per injection are listed to the right. Since the loss of sensitivity of hexestrol and enviroxime are similar in the first two experiments, it is clear from the last two experiments that hexestrol (a diphenol) is the primary source of the electrode inactivation. This is not surprising since phenols are well known to polymerize at electrode surfaces (5). It should also be noted from the data in Table 2 that enviroxime is capable of coating the electrode, albeit at a much slower rate.

The concentration dependence of the action of hexestrol is depicted in Figure 9. Here again, each data point represents one experiment, and the electrode was repolished in between each experiment. A roughly linear dependence is observed. The rate of coating was quite variable

TABLE 2

Compounds Injected (1 μg each)	% Decrease in Peak Height per Injection	
Enviroxime/Zinviroxime/Hexestrol	Enviroxime	14.3
	Hexestrol	14.4
Enviroxime/Hexestrol	Enviroxime	14.1
	Hexestrol	13.7
Hexestrol	Hexestrol	14.4
Enviroxime	Enviroxime	0.4

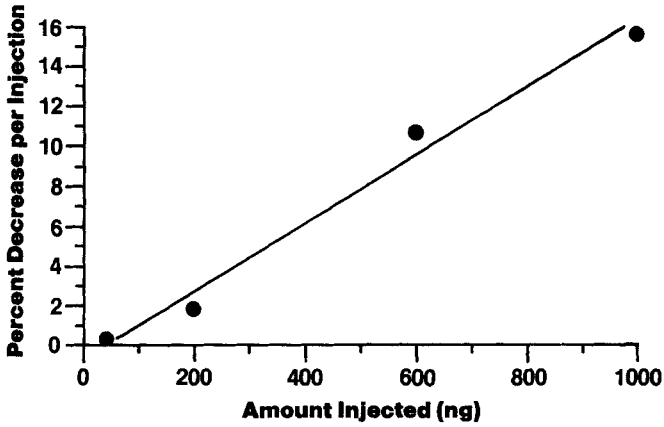


FIGURE 9. Concentration dependence of electrode coating by hexestrol. Coating determined by electrode response to zinviroxime.

(relative standard deviation = 27%) at the 200 ng level but reproducible at the 1 µg level. In order to minimize the coating of the electrode surface by hexestrol, or to regenerate coated electrodes, a variety of experimental approaches were tried. These are summarized in Table 3. The only approach found to be of signigicant value was to keep the amount of hexestrol injected low. It was possible in this case to do this without significantly degrading S/N. In addition, the similar electrochemical properties of hexestrol, zinviroxime, and enviroxime mean that hexestrol will reduce the effect on the analytes to sensitivity losses due to electrode coating. See for example Figure 10.

Studies of another benzimidazole structurally related to enviroxime manifested electrode coating in an additional way. When chromatographed under conditions similar to those for enviroxime, coating was seen at the rate of 0.4% per 300 ng injected. In addition, a dip in the baseline immediately after elution of the compound was observed (see Figure 11). The background current eventually returned to its initial level. The size of the dip is proportional to the amount of this compound injected. It was therefore attributed to reversible coating of the surface which strongly affected the background oxidation current. Such an effect has previously been observed (6). The reason for this reversibility was

TABLE 3

Experimental Approaches	Effective?
Switch E to −0.4 V for 10 min.	No
Let mobile phase "wash" surface overnight	No
Wash off-line with $CHCl_3$/acetone/CH_3OH	Partially
Switch from NaOAc to citrate/phosphate	Slightly
Add octyl sulfonate to mobile phase	No
Lower the amount injected	Yes

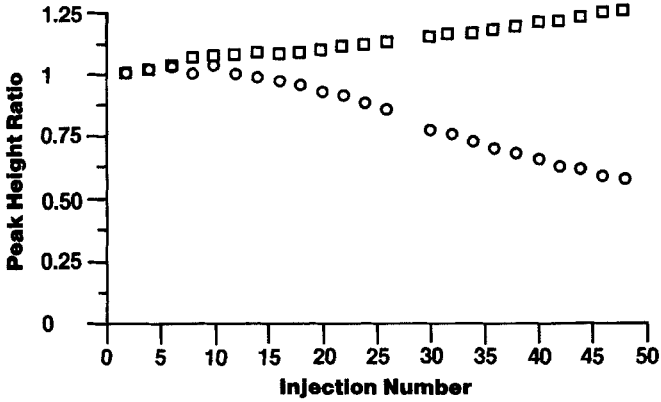


FIGURE 10. Peak height ratio for enviroxime upon repeated injection of a mixture of enviroxime and hexestrol. Key: 0 ratio to initial peak height, □ ratio to peak height of hexestrol.

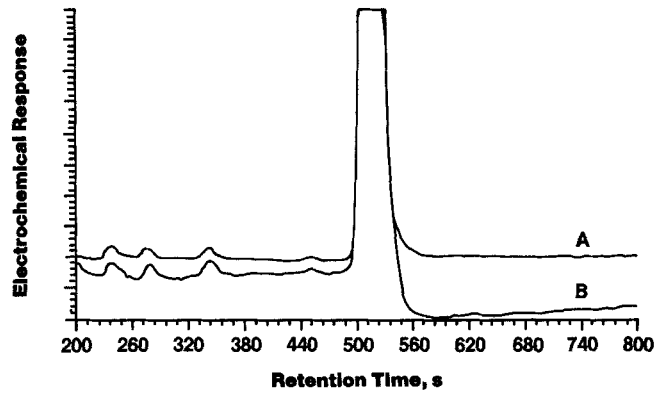


FIGURE 11. EC chromatograms of a benzimidazole. Chromatogram A was obtained at +0.86 V; The relative peak height was 64%. B was obtained at +0.90 V.

not known, and it is in contrast to the other example of coating encountered. The dip could be eliminated by lowering the oxidation potential (from +0.90 to +0.86 V).

The coating problems encountered with an octadecylenol analyte were noted above. It was presumed from the electrochemistry that octadecylalcohol is liberated during the oxidation of this molecule. Thus an attempt was made to reverse the loss of sensitivity by making large injections of wash solvent. This was unsuccessful, as were all other approaches tried for this compound (see Table 4).

In general we have been able to minimize electrode coating problems by lowering the amount of the compound injected, decreasing the detector potential or including an internal standard with electrochemical properties very similar to those of the analyte. These approaches have not always proven to be possible and successful. Greater success can probably be achieved though the use of pulsed EC detection for LC (7,8).

Late Eluters

Late-eluting peaks are strongly retained components from one injection, which elute during subsequent chromatograms. When samples are injected manually, the time between injections varies and the source and effect of late eluters is not always obvious. When an autosampler is used, the position of late eluters becomes reproducible and thus are more readily identified.

Late eluters will be observed at times with all types of LC detectors. Perhaps because of the superior sensitivity of EC detection,

TABLE 4

Experimental Approaches	Effective?
Lower detector potential	No
Change from glassy carbon to graphite/polymer	No
Inject slugs of wash solvent	No
Switch E to −0.65 between injections	No

we have more commonly observed late eluters with EC detection than with UV detection (see for example Figure 4). During the determination of enviroxime and zinviroxime in dog and rabbit liver homogenates and rabbit lung homogenates, many troublesome late eluters were observed. In the case of enviradene, late eluting peaks were observed in all physiological fluid extracts. Late eluters were a severe problem in the LC/EC determination of pergolide in feed discussed above.

In some simple cases it is possible to adjust the timing of injection such that late eluters elute in a non-critical portion of subsequent chromatogram. Waiting for the elution of all late eluters prior to making another injection is frequently not practical, even if elution is speeded by an increase in the organic modifier content of the mobile phase.

A very practical approach to removing late-eluting components is to use the well known technique of column-switching. For enviroxime and enviradene, a 3 cm column, mounted in a switching valve, was interposed between the pump and the 25 cm analytical column. The samples are injected onto the short column. After a time sufficient to allow the analytes and internal standard to elute from the 3 cm column onto the analytical column, the valve is automatically switched. The strongly retained components are then washed to waste using mobile phase and an auxiliary pump. Since the 3 cm column is washed with mobile phase there is very little reequilibration time when it is switched back into line. This type of system is ideal for running automated assays on large numbers of samples. Column switching produced cleaner chromatograms (compare Figure 12A and B), and compared with the other approaches mentioned above, increased sample throughput.

Interestingly, when column-switching was used during the determination of enviroxime in relatively clean biological extracts, fewer trace level interferences were seen. This resulted in an unexpected improvement in detection limits. The late eluters removed were small enough that their origin had not been determined.

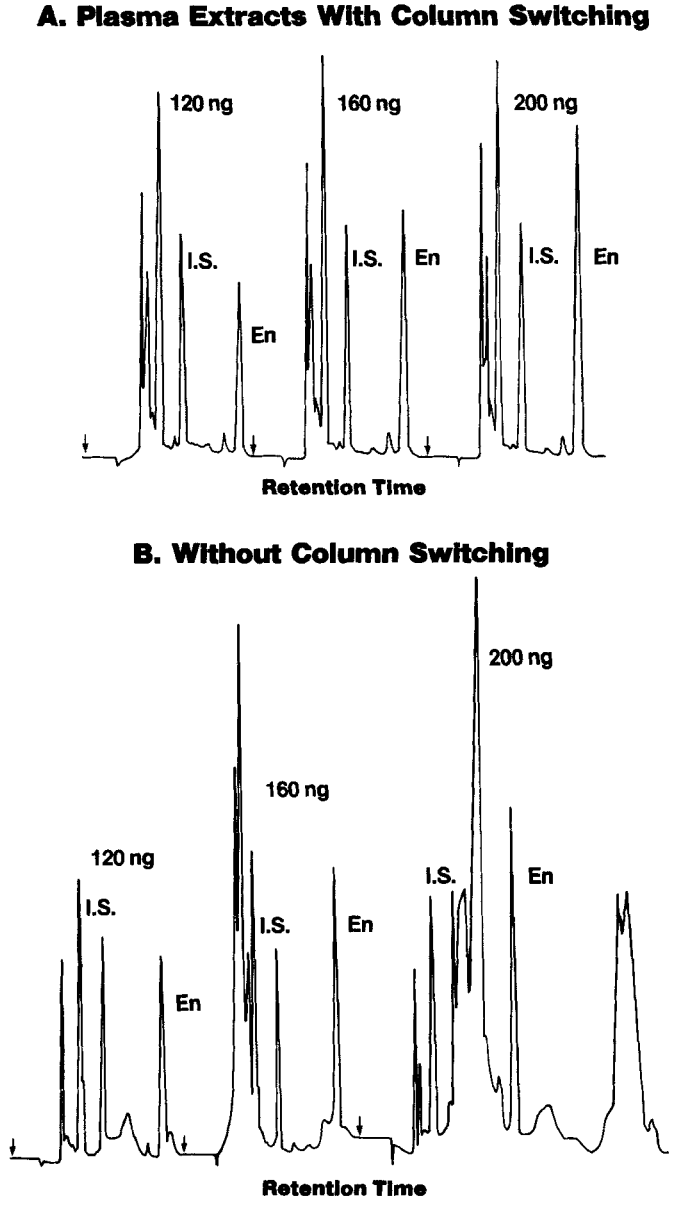


FIGURE 12. Chromatograms of extracts of plasma spiked with enviradene.

Temperature Dependence

A third common problem that can be encountered with the use of EC detection is the temperature dependence of amperometric detectors. This problem was originally recognized in our laboratory during lengthy unattended chromatography of enviroxime and zinviroxime-containing biological extracts (1). The EC responses to standards decreased dramatically when the ambient temperature decreased.

A detailed study was made in which several types of compounds, commonly determined by LC/EC, were chromatographed repeatedly with the detector temperature controlled over the range 0 to 50°C (2). Enviroxime and zinviroxime, the stilbestrols mentioned above, acetaminophen and some of its analogs, and several catecholamines were examined under typical assay conditions. Chromatographic response (peak height or area) of these compounds was observed to vary linearly as a function of temperature in some cases, and showed some upward curvature in others. The slope of these curves (taken at 22°C) ranged from a 1.5 to 2% change in EC response per °C for the catecholamines to greater than 8% per °C for zinviroxime.

The temperature dependence of amperometric EC detectors is due to the temperature dependence of 1) the diffusion coefficients of the analytes and 2) their rate of reaction at the electrode surface. Thus the changes in temperature influence both the number of molecules reaching the electrode surface and the fraction which are oxidized or reduced there. The contribution of diffusion was estimated by obtaining dependences with the working electrode potential high enough to assure diffusion-limited oxidation. Dependence of about 2% per °C or below were observed for the stilbestrols and acetaminophen and its analogs under such conditions. The contribution of changes in the rate of the electrode reaction to the overall temperature dependence increased exponentially as the detector potential was decreased, beginning about +75 mV above the hydrodynamic $E_{\frac{1}{2}}$ of the analytes.

A number of approaches are available which when used separately or in combination can decrease temperature dependences to insignificant levels. These are summarized in Table 5. The first is to thermostat the

TABLE 5

1. Thermostat detector
2. Thermostat column/increase flow rate
3. Operate well past $E_{1/2}$
4. Choose a good internal standard

EC detector, but this is not ordinarily convenient. The second is to thermostat the chromatographic column. Because of the dynamics of heat transfer in the EC detector cell block, thermostatting the column decreased the observed temperature dependences of acetaminophen and its analogs by a factor of 4. A third approach to minimizing EC temperature dependence is, whenever posssible, to operate well past the $E_{1/2}$ of the analyte, so that diffusion alone contributes to temperature dependence. Finally, the use of an internal standard with similar diffusional and electrochemical properties will largely compensate for temperature induced changes in response.

Baseline Instabilities

During initial assay development for DES and enviroxime, the background current of the glassy carbon working electrode oscillated erratically and gradually but continuously increased. Repolishing the electrode reduced the background to its initial level. Since EDTA was commonly added to mobile phases used in determination of penicillamine and catecholamines, it was added to mobile phases used for DES and enviroxime. With this addition the background current was much more stable, both in terms of long-term drift and medium-term oscillations. Metal ions leached from the steel LC components or from the silica backbone of the column are presumably reponsible for the baseline instabilities, but the mechanism of the phenomenon is not known. Use of phosphate/citrate buffers in the mobile phase have some of the same effect as EDTA.

A second contributor to long-term drift is electrode coating by constituents of the mobile phase. Analagous to the coating by analytes discussed above, this is most frequently a problem at high oxidation

potentials. During the determination of enviroxime, it was found that the use of a reagent grade methanol in the mobile phase caused a 50% loss of sensitivity after only a few days of continuous use. Two manufacturers' distilled-in-glass methanols gave better results, but one (Burdick and Jackson) was clearly superior to the other. This assay is probably particularly sensitive to coating effects, since the oxidations are not at diffusion-limited conditions. Nevertheless, the benefits to be gained from use of the best available reagents for a given determination should be carefully considered.

CONCLUSION

The versatility of EC detection for LC has been demonstrated in our laboratory by its application to the determination of over a dozen compounds in biological matrices. Its durability has been proven by the successful assay of over 15,000 samples. Several problems which were encountered during routine use of the electrochemical detector have been investigated. A number of practical ways of overcoming these difficulties were evaluated, and in most cases the problems were surmountable.

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