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EXPERT SYSTEM FOR PHARMACEUTICAL ANALYSIS

II. RELATIVE CONTRIBUTION OF AND RULE VALIDATION FOR AMPEROMETRIC DETECTION (OXIDATION MODE)

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SUMMARY

The expert system predicted correctly whether a drug could be detected with an amperometric detector (oxidation mode) in 93% of the compounds investigated. The validation was performed for drugs possessing a low molar extinction coefficient and for pharmaceuticals containing only small amounts of drugs. For 50% of this group of drugs, the amperometric detector could be used and the sensitivity was significantly increased in comparison with UV detection. The remaining groups of drugs, *i.e.*, for those for which neither UV detection nor the amperometric detector offered a solution, are also reported, together with some UV-inactive drugs that could be determined only by amperometric detection.

INTRODUCTION

Expert systems as a part of artificial intelligence will become a powerful tool in analytical chemistry[1,2] and also in column liquid chromatography[3,4]. They are software products that offer intelligent advice for problems requiring some expertise. Since the development of high-performance liquid chromatographic (HPLC) methods for pharmaceutical and biomedical analysis is often rather complex, it is our intention to build an expert system that takes intelligent decisions about the selection of parameters for chromatographic analysis of drugs.

The design of this expert system consists of different decision nodes that are structured in the form of a decision tree (Fig. 1). The expert system tries to find an initial selection of parameters in the space of possibilities by reasoning from one node to another. It first selects the appropriate detection system. This decision depends on the required sensitivity and selectivity, and on the characteristics of the substances to be quantified. Since an universal detector with sufficient sensitivity is not available in liquid chromatography, three native detection systems are incorporated into the expert system: UV, amperometric and fluorescence detection. This series of detectors is chosen because their properties complement each other: the range of application

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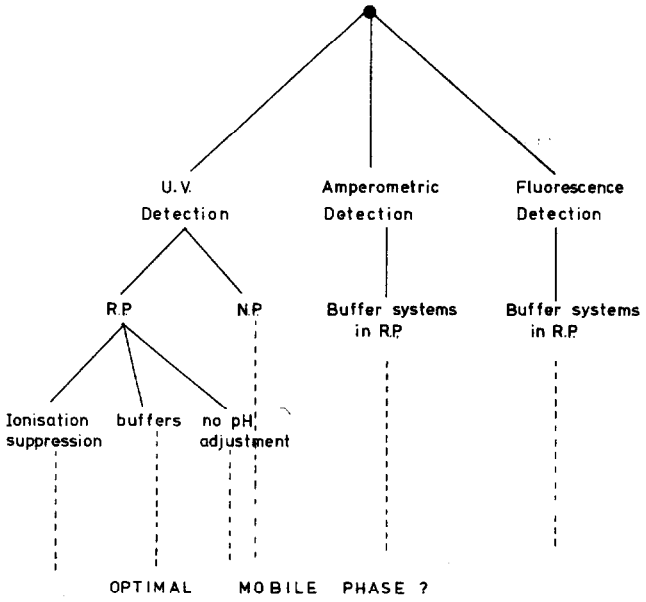


Fig. 1. An example of a decision tree in HPLC. R.P. = Reversed phase; N.P. = normal phase.

is the largest for UV, the selectivity for fluorescence detection and the sensitivity for amperometric detection[5–10].

The selection of a suitable detector has implications for the composition of the chromatographic system: amperometric and fluorescence detectors are preferably used with buffer systems, while this is rarely necessary with UV detection. In our strategy the stationary phase is always a cyanopropyl bonded phase[11] so that the expert system only decides in the second node whether a reversed phase, normal phase or buffers have to be used. Afterwards one can optimize the mobile phase composition. The scope of this article is restricted to the first node: UV *versus* amperometric detection.

The construction of an expert system involves three important stages. First the knowledge base used by the "expert" must be set up. This consists of facts (data) and rules. The second step is the validation of these rules and the last one consists in the implementation of the validated rules. The aim of this work is to develop a list of electro-oxidizable functions so that the system is able to decide whether a compound can be oxidized and detected with an amperometric detector by looking at the presence of certain functional groups in the molecule. This is only necessary if UV detection does not yield acceptable results[12].

In the expert system, UV detection is preferred and amperometric detection is used only when UV detection is not sufficient. In order to make an assessment of the contribution of amperometric detection to the detection of drugs with a low UV response, the validation is performed here on drugs possessing a low molar extinction coefficient and on pharmaceuticals containing small amounts of drugs. This permits the determination of the relative contribution of amperometric detection compared to UV detection: in which cases does amperometric detection offer a solution when

UV detection does not? For all redox active compounds of the test set selected, a voltammogram was recorded and the gain in sensitivity using an amperometric *versus* a UV detector was determined.

EXPERIMENTAL

Instrumentation

The HPLC apparatus included a Varian 8500 liquid chromatograph equipped with a Valco injector (50 μl). Two detection systems were coupled in series: a Varian UV detector with fixed wavelength 254 nm (optical pathlength 1 cm, cell volume 8 μl) and an LKB 2143 amperometric detector (glass–carbon electrode, cell volume 5.5 μl). A two-pen Kipp and Zonen BD9 recorder was used.

An HPLC system consisting of a Varian 5000 liquid chromatograph equipped with a Rheodyne injector (100 μl) and a Hewlett-Packard 1040 A diode-array detector (optical pathlength 0.6 cm, cell volume 4.5 μl) was used as a variable wavelength detector and also to monitor the spectra of all the compounds investigated. The absorptions were calculated with an HP 85 B and the chromatograms were recorded with a Varian Vista CDS 401 instrument.

Chromatographic conditions

A (250 mm × 4 mm I.D., particle size 5 μm) LiChrosorb CN column was used with a mobile phase of acetonitrile–phosphate buffer (pH 3, ionic strength = 0.05) (40:60) containing 0.001 *M* sodium chloride. The buffer solution was filtered through a 0.2-μm membrane filter and the mobile phase was thoroughly degassed before use. The flow-rate was 1 ml/min and all experiments were performed at room temperature.

Chemicals and reagents

The stock solutions were prepared in the mobile phase and stored at 4°C. Standard solutions were diluted in the mobile phase and prepared fresh daily. All drugs were of pharmacopoeial purity. Acetonitrile (Merck, Darmstadt, F.R.G.) was of liquid chromatographic grade. Phosphoric acid, sodium dihydrogenphosphate and sodium chloride were also obtained from Merck.

Software

The software for the expert system is being developed with an expert system tool kit called KES on an Apollo workstation. This will be described elsewhere[13].

RESULTS AND DISCUSSION

There is a great body of empirical information concerning amperometric detection in HPLC. In general, one can state that the electrochemical behaviour of a compound depends primarily on the molecular structure, but that it can be influenced by other parameters such as the mobile phase composition (pH, organic modifier, etc.) and the amperometric cell used (design, material from which the working electrode is made, etc.).

All the experiments were performed using the same eluent, for reasons described in Part I of this series[12]. An amperometric detector equipped with a thin-

layer cell and a glass–carbon electrode as working electrode is used, since its characteristics as a detection for the oxidation of organic compounds have been described in detail[10,14]. The eluent was selected first because it is compatible with all detection systems of the expert system, and secondly since its use with a cyanopropyl bonded phase can be considered as a general chromatographic system in drug analysis[12].

In earlier work[12] we reported that compounds containing a phenol, a primary or secondary aromatic amine, an aromatic methoxy or ethoxy or a thiol function are detectable by amperometric detection. Based on our experiments in the search for electroactive drugs, this list of electro-oxidizable functions can be expanded by adding the following functions: a phenothiazine sulphur, a secondary or tertiary aliphatic amine, piperazine and dioxazine. Exceptions are tertiary alicyclic amines (such as dipipanol, diphenylpyraline and procyclidine) and secondary aliphatic amines, bonded to two atoms that each form a double bound with another atom (as in diazepam, amobarbital, cloxacillin and dicloxacillin) (Table I).

The molecular structure of the drug, for which the expert system is asked to select a suitable detection system, is scanned for the presence of at least one oxidizable

TABLE I

LIST OF OXIDIZABLE FUNCTIONS

R is radical. X, Y, V and W are variables.

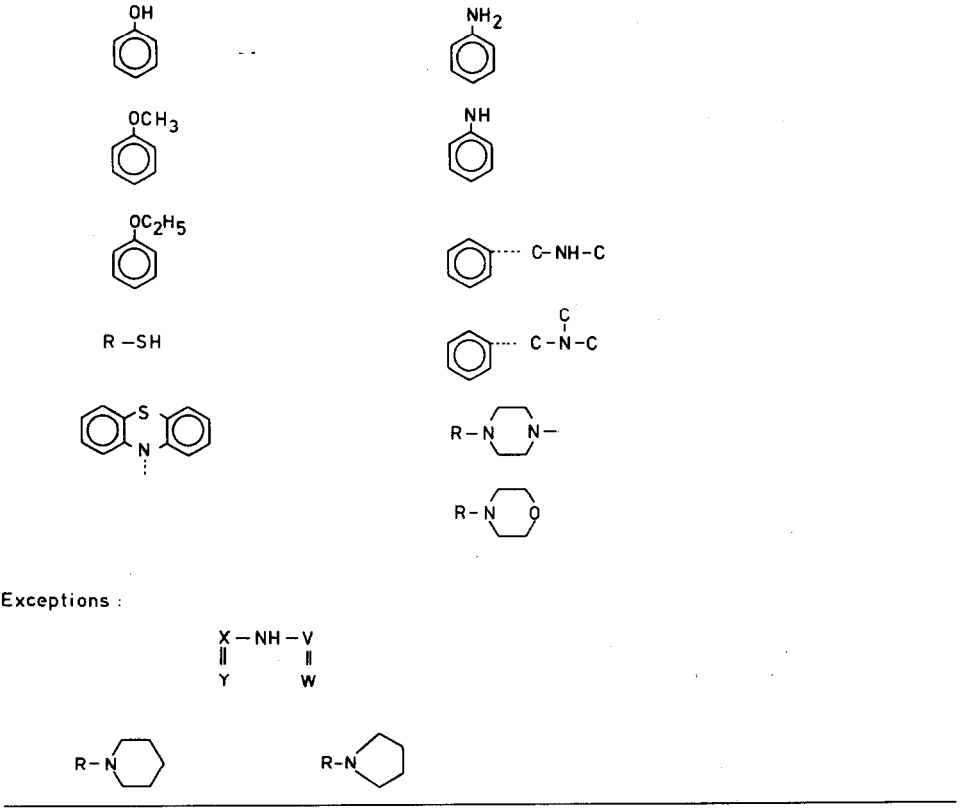
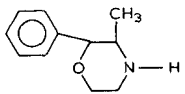
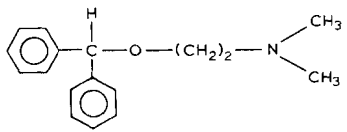
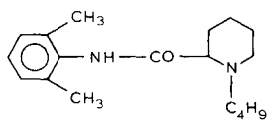
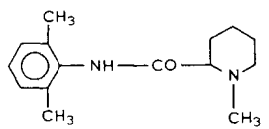
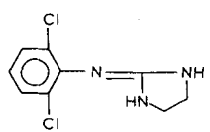


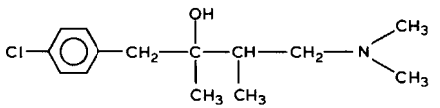
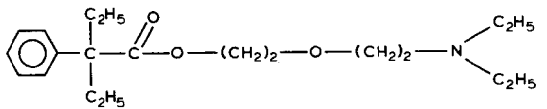
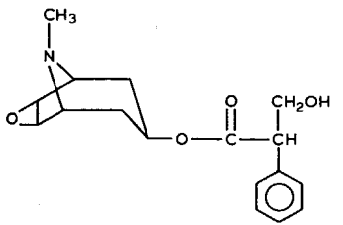
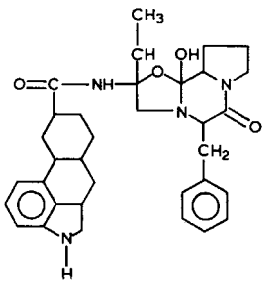
TABLE II

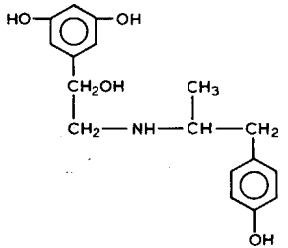
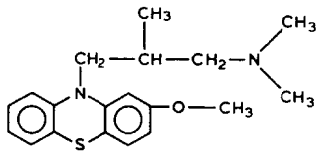
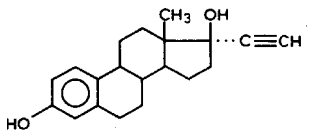
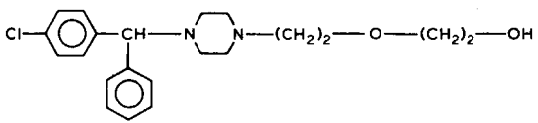
COMPARISON BETWEEN MINIMUM DETECTABLE QUANTITIES WITH UV DETECTION AND ELECTROCHEMICAL DETECTION (ED) FOR DRUGS POSSESSING LOW MOLAR EXTINCTION COEFFICIENTS

Compound	*Molecular structure*	*k'*	*MDC* (ng o/c)*			*Applied potential (V)*	*Gain** ED*
			UV 254 nm, l = 1 cm	*λ_{max}, l = 0.6 cm*	*ED*		
(a) Theoretical prediction of a positive oxidative response is confirmed by the experiments							
Phenmetrazine		1.5	769	1155	26.5	1.2	29
Diphenhydramine		2	599	847	128.2	1.1	4.7
Bupivacaïne		1.8	643	1156	24.6	1.2	26
Mepivacaïne		1.7	455	790	17.7	1.2	25.7
Clonidine		1.8	910	733	8.6	1.2	85.2

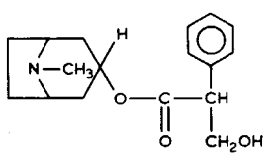
(*Continued on p. 6*)

TABLE II (*continued*)

Compound	*Molecular structure*	*k'*	*MDC* (ng o/c)*			*Applied potential (V)*	*Gain** ED*
			UV 254 nm, l = 1 cm	*λ_{max}, l = 0.6 cm*	*ED*		
Clobutinol		1.8	1818	1653	69.4	1.1	23.8
Oxeladine		0.6	204	75.5	6.3	1.2	12
Scopolamine		1.1	1282	1960	50	1.1	25.6
Dihydroergotamine		1.5	253.2	173.8	2.7	1.1	64.4

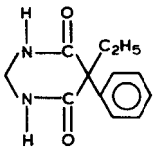
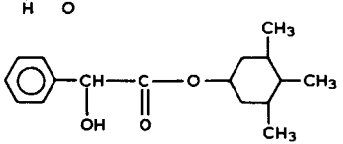
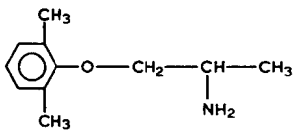
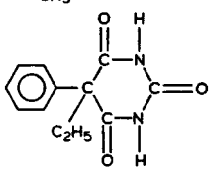
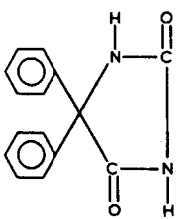
Fenoterol		1	376.8	118.5	1.1	1.1	107.7
Levomepromazine		2.4	7.8	14.5	0.4	1.2	19.5
Ethinylestradiol		0.7	468	113	1.1	1.1	102.7
Hydroxyzine		2	360	45.2	7.4	1.2	6

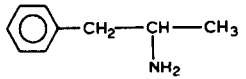
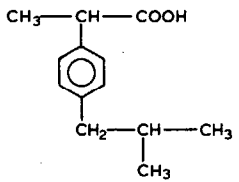
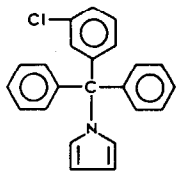
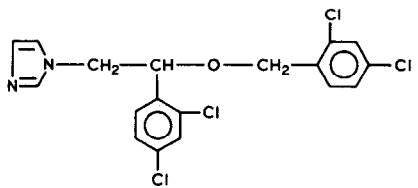
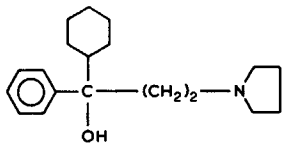
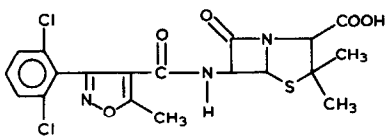
(b) Theoretical prediction of a negative oxidative response is confirmed by the experiments

Atropine sulphate		1	1124	–	–	–	–

(*Continued on p. 8*)

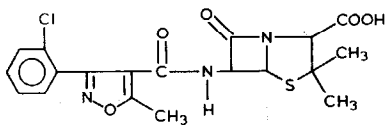
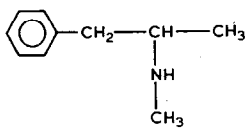
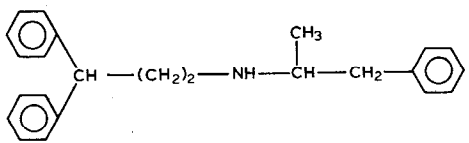
TABLE II (*continued*)

Compound	*Molecular structure*	*k'*	*MDC* (ng o/c)*			*Applied potential (V)*	*Gain** ED*
			UV 254 nm, l = 1 cm	*λ_{max}, l = 0.6 cm*	*ED*		
Primidone		0.4	487	–	–	–	–
Cyclandelate		0.7	709	–	–	–	–
Mexiletine		0.9	636	824	–	–	–
Phenobarbital		0.4	510	–	–	–	–
Phenytoïne		0.75	142	–	–	–	–

Amphetamine		0.8	622	–	–	–	–
Ibuprofen		0.6	420	580	–	–	–
Clotrimazol		1.6	352	503	–	–	–
Miconazol		1.75	1341	1143	–	–	–
Procyclidine		1.5	1667	–	–	–	–
Dicloxacillin		0.5	231	–	–	–	–

(*Continued on p. 10*)

TABLE II (*continued*)

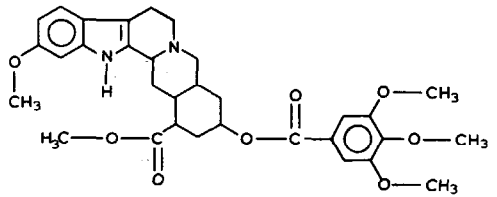
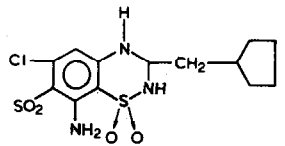
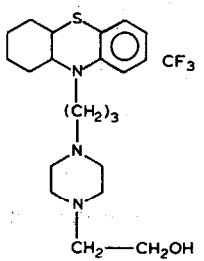
Compound	*Molecular structure*	*k'*	*MDC* (ng o/c)*			*Applied potential (V)*	*Gain** ED*
			UV 254 nm, l = 1 cm	*λ_{max}, l = 0.6 cm*	*ED*		
Cloxacillin		0.45	155	–	–	–	–
(c) Theoretical prediction of a positive oxidative response is in contradiction with the experiments							
Metamphetamine		0.8	656	–	–	–	–
Prenylamine		1.9	595	–	–	–	–

* Minimum detectable concentration (on column).

** The factor gain in sensitivity with an amperometric detector.

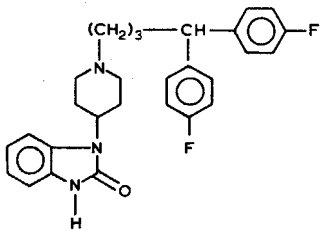
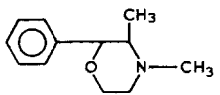
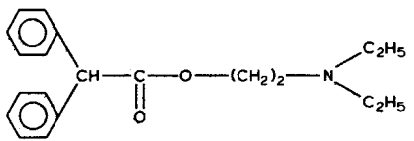
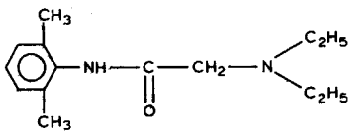
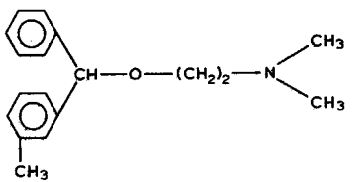
TABLE III

COMPARISON BETWEEN MINIMUM DETECTABLE QUANTITIES OBTAINED WITH UV DETECTION AND ELECTROCHEMICAL DETECTION FOR PHARMACEUTICALS CONTAINING SMALL AMOUNTS OF DRUGS

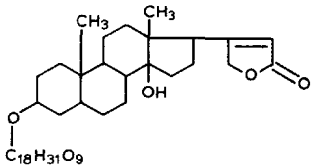
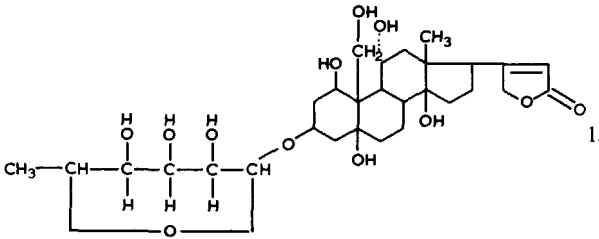
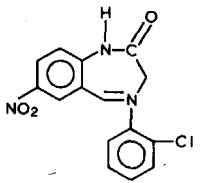
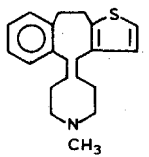
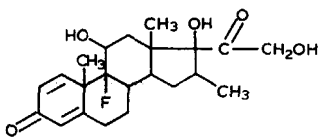
Compound	*Molecular structure*	*k'*	*MDC* (ng o/c)*			*Applied potential (V)*	*Gain** ED*
			UV 254 nm, l = 1 cm	*λ_{max}, l = 0.6 cm*	*ED*		
(a) Theoretical prediction of a positive oxidative response is confirmed by the experiments							
Reserpine		2.5	51.2	60.1	4.2	0.9	14.3
Cyclopenthiazide		1	41.5	19.4	19.6	1.2	1
Fluphenazine		2.6	22.2	34.3	2.7	1.1	8.2

(*Continued on p. 12*)

TABLE III (*continued*)

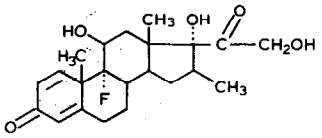
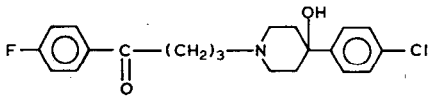
Compound	*Molecular structure*	*k'*	*MDC* (ng o/c)*			*Applied potential (V)*	*Gain** ED*
			UV 254 nm, l = 1 cm	*λ_{max}, l = 0.6 cm*	*ED*		
Pimozide		2.1	321.1	119.1	10.1	1.2	11.8
Phendimetrazine		1.4	750	1587	16.7	1.2	45
Adiphenine		1.8	909	1587	36	1.1	25.3
Lidocaïne		1.7	403	–	1.9	1.2	212
Orphenadrine		2	563	847	75.2	1.1	7.5

(b) Theoretical prediction of a negative oxidative response is confirmed by the experiments

Digitaline		0.5	741.3	–	–	–	–
Ouabaïne		1.75	972.4	–	–	–	–
Clonazepam		0.6	17.8	16.8	–	–	–
Pizotifen		1.6	28.6	28.4	–	–	–
Betamethazon		0.5	16.4	19.8	–	–	–

(Continued on p. 14)

TABLE III (*continued*)

Compound	*Molecular structure*	*k'*	*MDC* (ng o/c)*			*Applied potential (V)*	*Gain** ED*
			UV 254 nm, l = 1 cm	*λ_{max}, l = 0.6 cm*	*ED*		
Dexamethazon		0.5	15	18.3	–	–	–
(c) Theoretical prediction of a negative response is in contradiction with the experiments							
Haloperidol		1.35	36.6	46.4	3.5	1.0	10.4

* Minimum detectable concentration (on column).

** The factor gain in sensitivity with an amperometric detector.

function, in order to predict whether it can be detected by amperometric oxidation or not. These decisions were compared with experimental results for 43 drugs. Since at the same time we wanted to assess the relative contribution of amperometric detection, *i.e.*, to find in which cases it solves detection problems that cannot be solved by UV detection, the drugs selected all possessed a low UV response.

The test set comprised two different groups. The first one was obtained from an atlas of UV spectra of drugs[15]. From all compounds possessing a molar extinction coefficient lower than 1000 (measured in methanol at the absorption maximum) one

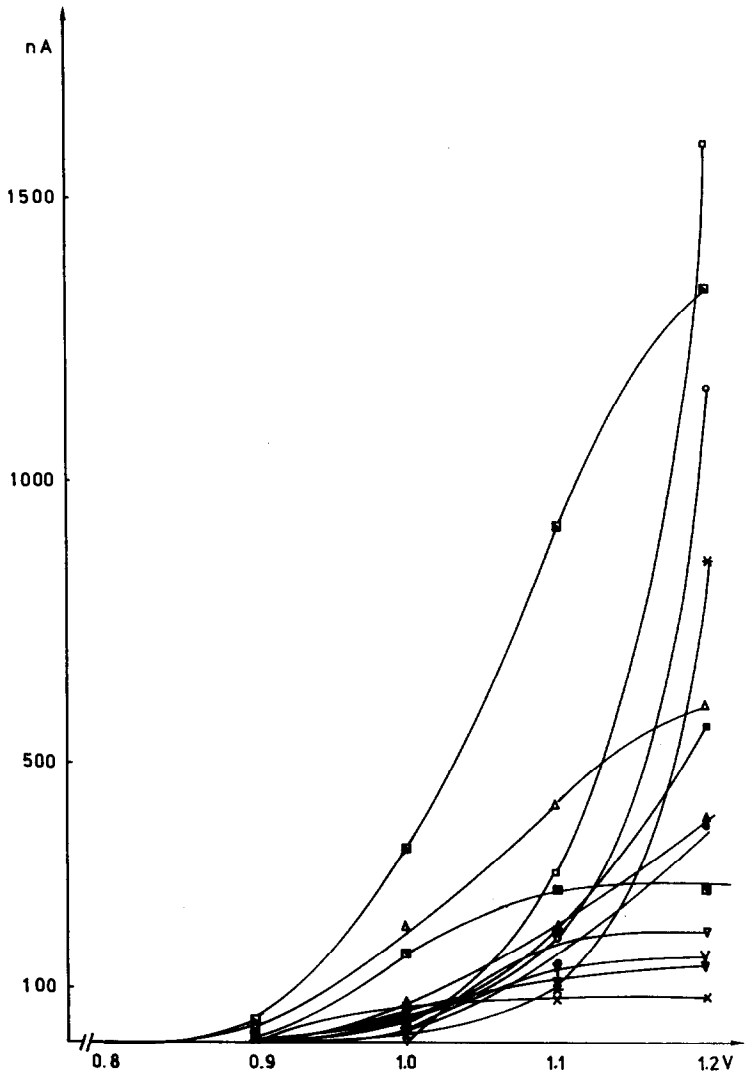


Fig. 2. Voltammogram of compounds possessing low molar extinction coefficients: ●, phenmetrazine; ×, diphenhydramine; ▲, bupivaceine; ■, mepivacaine; ○, clonidine; ▼, clobutinol; □, oxeladine; ▽, scopolamine; △, phendimetrazine; ◹, hydroxyzine; ✳, lidocaine; ◹, radiphenine; Y, orphenadrine. The concentration of all the solutes was 10 ppm, except for lidocaine, 1 ppm.

third was selected at random. These 28 drugs, listed in Table II, were used to verify the decisions made by the expert system. The second group comprised drugs that are usually present in low concentrations in pharmaceuticals, *i.e.*, less than 1 mg per formulation unit. These drugs were found in the Belgian drug compendium[16]. Half were selected for study (Table III). The amperometric detector is set at 1.2 V by the operator at a range of 200 nA full scale. When an electrochemical signal was obtained under these conditions the substance was considered to be electroactive. For each such compound a voltammogram was recorded. The potential just before the start

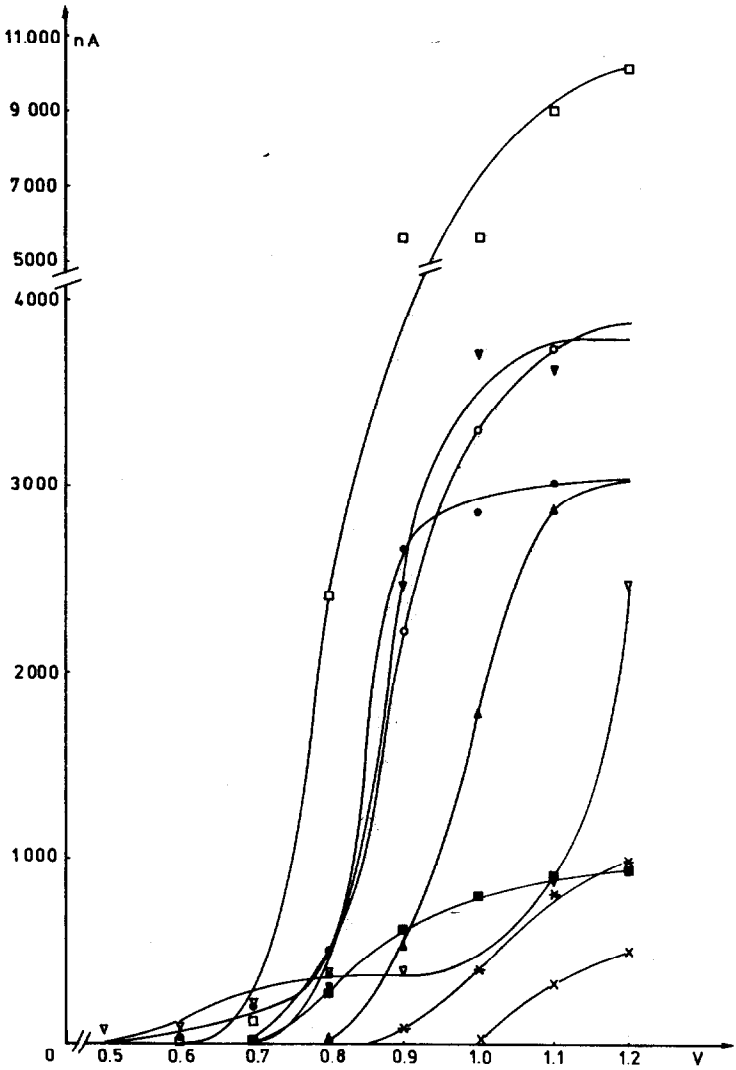


Fig. 3. Voltammogram of compounds present in low concentrations in pharmaceuticals: ●, reserpine; ×, cyclopenthiazide; ▲, haloperidol; ✳, pimozide; ○, dihydroergotamine; □, fenoterol; ▼, fluphenazine; ▽, levomepromazine; ■, ethinylestradiol. The concentration of all the solutes was 10 ppm, except for ethinylestradiol, 1 ppm.

of the limiting-current plateau was applied since at this point the signal-to-noise ratio is the most advantageous. At this potential the amount of drug providing a signal equivalent to 20 nA is considered to be the minimum detectable concentration (MDC-ED) and is compared with the amount of drug giving a signal equivalent to 0.002 absorption units (MDC-UV) (Tables II and III). The lowest MDC-UV of the two UV detection systems mentioned is compared with the MDC-ED value to calculate the gain in sensitivity when an amperometric detector is applied (Tables II and III).

For 15 of the 28 compounds with low molar extinction coefficients (Table II) the expert system predicted a positive electrochemical response. The experiments showed that for 13 drugs the decision was correct (Fig. 2); for the other 2 compounds however no electrochemical signal was obtained. These exceptions are secondary and tertiary amines, namely prenylamine and methamphetamine. Analogous structures such as pro- and nortriptyline, propranolol and pindolol are electroactive[12].

For the 13 drugs listed in Table II, no response was expected and indeed not obtained. Barbiturates and primary aliphatic amines represent the most important classes of drugs in this group. A second possible reason for an expected low UV

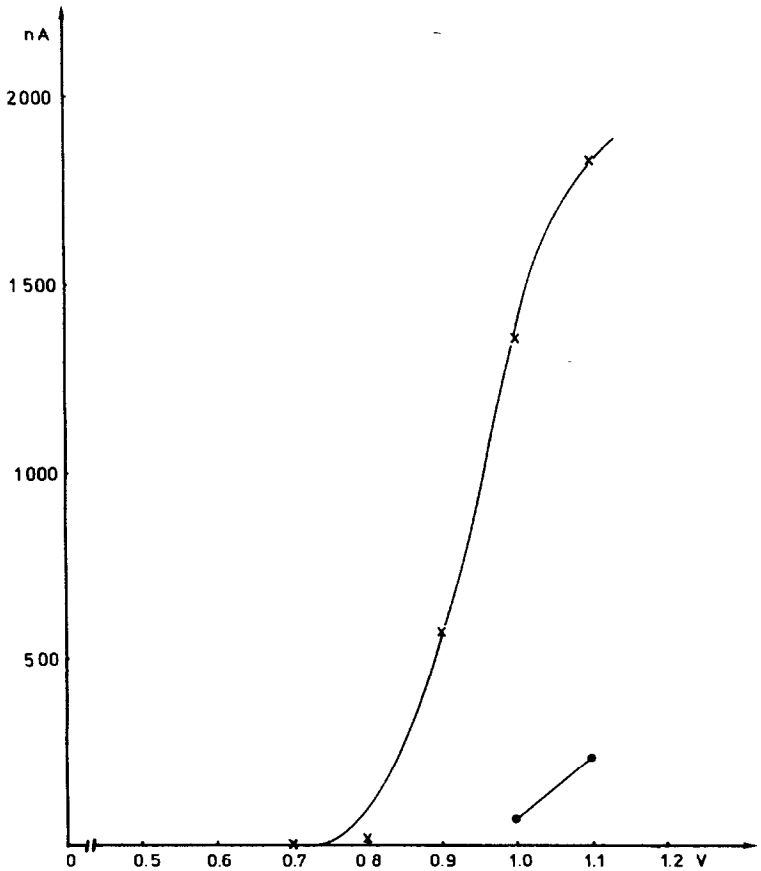


Fig. 4. Voltammogram of UV-inactive compounds: ●, piperazine (5 ppm); ×, penicillamine (0.5 ppm).

response is a low concentration. The utility of amperometric detection was also investigated for this group. For 9 compounds out of the 15 a voltammogram was recorded (Fig. 3). In only one case, namely haloperidol, an unexpected positive electrochemical signal was obtained. This is probably due to the position of the alcohol function near the aromatic ring (pseudo phenol function). Although the molar extinction coefficients are not low, the gain in sensitivity was significant for the amperometric detector, especially for phenolic structures such as fenoterol and ethinylestradiol (Table IIIa and c). For cyclopenthiazide however no improvement was obtained with an amperometric detector. Relatively low MDC-ED values were obtained for reserpine, fluphenazine, dihydroergotamine, haloperidol and levomepromazine. For the drugs listed in Table IIIb, no electroactive response was obtained.

CONCLUSIONS

Looking at the total test set, it is seen that the expert system predicted correctly whether a compound could be detected with an amperometric detector with the general chromatographic conditions used in 40 of the 43 cases investigated (93%). The percentage of correct decisions is acceptable for use in expert systems in the sense that an human expert would certainly not score better.

Amperometric detection offers a possible solution for 50% of this group of drugs with a low UV response since the sensitivity is nearly always enhanced. For some compounds that are not UV active at all, such as piperazine and penicillamine (Fig. 4), amperometric detection also offers a solution.

Phenols, primary aromatic amines, phenothiazines and indoles are most suitable for amperometric detection in the oxidation mode. The drugs with detection problems, *i.e.*, insufficient UV and amperometric response, are mainly barbiturates, corticosteroids, male hormones, amphetamines and analogous compounds and some antibiotics such as cloxacillin.

As a general conclusion one can state that amperometric detection is useful for pharmaceutical and biomedical analysis, but that for some compounds other detection systems must be investigated if the sensitivity and/or the selectivity is to be enhanced.

In Part I of this series in which we developed the rules[12], 72 electroactive substances were investigated. Applying the rules as described here to these substances and the others used in this study we find that of the 94 cases where the drug is electroactive this is recognized by the rules, except in the cases of haloperidol, prenylamine and metamphetamine.

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