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EXPERT SYSTEM FOR PHARMACEUTICAL ANALYSIS

I. SELECTION OF THE DETECTION SYSTEM IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS: UV *VERSUS* AMPEROMETRIC DETECTION

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SUMMARY

The usefulness of amperometric detection in pharmaceutical analyses was investigated for different groups of drugs. The UV response at 254 nm and that at the absorption maximum of the solute were compared with the electrochemical signal obtained. The minimum detectable concentration (nanograms on-column) of each substance is reported for the three different detection systems. This comparison was performed for 72 drugs (local anaesthetics, antipyretics, tricyclic antidepressants, sulphonamides, sex hormones, beta-adrenoceptor blocking agents, phenothiazines, alkaloids, diuretics and penicillins). The median limit of detection of the amperometric detector (see definition in the text) is 1.0 ng on-column and the median gain in sensitivity, compared with UV detection is 22.5.

INTRODUCTION

An expert system has been defined as the embodiment of a knowledge-based component from an expert skill in such a form that the system can offer intelligent advice or take an intelligent decision about a processing function[1]. It is our intention to build an expert system that helps the analyst in taking decisions about method selection [high-performance liquid chromatography (HPLC), gas–liquid chromatography (GLC) and UV spectroscopy] and development (for HPLC: selection of the detector, choice between reversed or normal phase) in pharmaceutical and biomedical analysis[2].

Laboratories that are required to carry out many different analyses spend a lot of time on method development, which explains the interest that has arisen in recent years in formal optimization procedures for chromatographic separations, particularly among pharmaceutical analysts. However, optimization is only the last step in the method development process, being preceded by the selection of the outline of the method. There are two aspects to this. The first is the development of a strategic approach to method selection, which was developed by Hoogewijs and Massart[3]. An

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essential element in this approach was the selection of a cyanopropyl bonded phase as a single stationary phase suitable for the chromatography of nearly all basic drugs. This was extended later[4] to all pharmaceutical substances. It was stated that use of this single phase with a limited number of mobile phases is a good approach for any drug analysis. The second step is the incorporation of this strategy into an expert system. However, additional elements are then needed and one of these is the selection of the detector. Indeed, this has implications for the selection of the mobile phase by the expert system: for instance, amperometric detectors are preferably used in reversed-phase systems and this implies the use of buffer solutions in the mobile phase.

UV detection is the most universal detection system in HPLC. However, other detection systems are often necessary, sometimes to extend the application range and sometimes to improve the limit of detection and the selectivity[5–9]. The limit of detection is the minimum concentration of solute, in mass per unit volume, passing through the detector that can be discerned from the noise. Detectors sensitive only to some property of the solute are defined as selective detectors[7]. The selection of the wavelength (UV detection), the excitation and the emission wavelength (fluorescence detection) or the potential (amperometric detection) determines the selectivity. In this work, the amperometric detector was selected as the second detection system. Its advantages were reported by Kissinger, Shoup and co-workers[10–12], pioneers in the field of electrochemical detection in liquid chromatography, and confirmed by other workers[13–17].

The first important application in liquid chromatography with electrochemical detection was the use of an amperometric detector for the assay of catecholamines in biological material[11,15,18], which has led to an exponential development of electrochemical detection during the last few years. The technique has received growing interest in biomedical applications because, owing to the higher selectivity, the sample clean-up can be simplified[15]. Simultaneously, better electrochemical detectors are being developed[19–27], and the application range of redoxactive substances was extended using derivatization techniques[15,17,28,29].

In order to study the application range and the gain in sensitivity of amperometric detection *versus* UV detection, ten groups of drugs were monitored by UV and amperometric detection systems, using the same chromatographic system. This comparative study was performed for local anaesthetics, antipyretics, tricyclic antidepressants, sulphonamides, sex hormones, beta-adrenoceptor blocking agents, phenothiazines, alkaloids, diuretics and penicillins. These groups were chosen partly on the basis of a review by Stulik and Pacakova[15], who cited applications to paracetamol[30–33], morphine[34], apomorphine[35], tricyclic antidepressants[36], phenothiazines[37], amoxicillin[38], diethylstilbestrol[39–41] and estriol[42,43]. The other compounds were selected because they possess a phenol or a primary amine function. Within one pharmacological group, some substances have modified phenol or amine functions, which are interesting for investigating the influence of the substitution on the electrochemical properties.

Expert systems consist of a knowledge base and a set of rules, which together constitute the so-called domain expertise (the knowledge of the expert). Although a lot of knowledge has accumulated in the literature concerning electrochemical detection, it is not present in a form that is optimal for expert systems. In the literature one finds many studies concerning a single or a few drugs and possibly their metab-

olites[44–54]. However, a systematic comparison of detection systems for compounds of pharmaceutical interest does not exist, as far as we know. It was our intention to make this comparison and thereby to bring the domain expertise into a form suitable for use in expert systems.

EXPERIMENTAL

Apparatus

A Varian 8500 liquid chromatograph, equipped with a Valco injector (50 μl) and a Varian UV detector with a fixed wavelength of 254 nm (optical path length 1 cm, cell volume 8 μl), coupled in series with an LKB 2143 amperometric detector (glass–carbon electrode, cell volume 5.5 μl) was used. A Kipp and Zonen BD9 two-pen recorder was used to record the chromatograms.

A Varian 5000 liquid chromatograph, equipped with a Rheodyne injector (100 μl) and a Hewlett-Packard 1040A diode-array detector (optical path length 0.6 cm, cell volume 4.5 μl), was used for UV detection. The chromatograms were recorded with a Varian CDS 401 instrument and the absorption values were calculated with a Hewlett-Packard 85B.

Chromatographic parameters

LiChrosorb CN column (250 × 4 mm I.D., particle size 5 μm) was used. The mobile phase was acetonitrile–phosphate buffer (pH 3, μ = 0.05) (40:60), containing 0.001 *M* NaCl. All experiments were performed at a flow-rate of 1 ml/min and at ambient temperature.

Reagents

All drugs were of pharmacopoeial purity. The stock solutions and the appropriate standard solutions were dissolved in the mobile phase and stored at 4°C between use. Acetonitrile was of liquid chromatographic grade. Phosphoric acid, NaH_2PO_4, sodium dihydrogen phosphate, sodium chloride and acetonitrile were purchased from Merck (Darmstadt, F.R.G.).

RESULTS AND DISCUSSION

The selection of detection systems by the expert system is based on the following principles: native detection is preferred to pre- and post-column derivatization, because it is less complex; pre- and post-column derivatization are considered as last resort possibilities; UV detection at 254 nm is the first possibility that is to be considered. If this is not suitable, UV detection at the absorption maximum of the solute is investigated. If this does not yield acceptable results, the amperometric detection system is to be investigated as a third possibility. It was our intention to study subsequently the incorporation of fluorescence and coulometric detection[55–57] in the knowledge base, so that the expert system can offer intelligent advice about the selection of the detection system. In this work we therefore studied only two types of detector, namely UV and amperometric.

For all the drugs investigated, the selectivity and the limit of detection of the amperometric detector were determined. A voltammogram was recorded for each

compound, and a potential just before the start of the limiting current plateau was chosen. At this potential the amount of drug providing a signal equivalent to 20 nA is considered to be the minimum detectable concentration (MDC-E) and is compared with the amount of drug giving a signal equivalent to 0.002 absorption units (MDC-UV). The most favourable, *i.e.*, the lowest MDC of the two UV detection systems mentioned, is then compared with the MDC-E to calculate the gain in sensitivity when an amperometric detector is applied. The same chromatographic system was applied for the three detectors, so that equal k' values were obtained. As the aim of this work was not the selection of optimal mobile phases for each different solute, the same eluting agent was used for all chromatograms. The retention time, the applied potential, the MDC values for the three mentioned detection systems and the gain in sensitivity of the amperometric *versus* UV detector are given for all the investigated drugs in Table I. Typical chromatograms are shown in Fig. 1. The potential in the recorded voltammogram where the first oxidation signal was obtained is important for the selectivity. Indeed, the lower the voltage used, the less other substances will be oxidized and also the lower the background is (Fig. 2).

TABLE I

COMPARISON BETWEEN MINIMUM DETECTABLE CONCENTRATIONS (MDC) WITH UV DETECTION AND ELECTROCHEMICAL DETECTION

l = Optical path length of the UV detector (cm)

Group	*Compound*	*MDC (ng on-column)*			*Gain* (ECD)*	*k'*	*Applied potential (V)*
		UV (254 nm) (l = 1)	*UV $\lambda_{max.}$ (l = 0.6)*	*ECD*			
Local anaesthetics	Amylocaine	131	18.7	5.3	3.5	1.5	1.2
	Benzocaine	14.7	7.2	0.2	36	0.6	1.2
	Procaine	46.8	12.6	0.5	25	1.35	1.2
	Tetracaine	255	12.4	1	12.4	1.6	1.2
	Lidocaine	403	–	1.9	212	1.7	1.2
Antipyretics	Acetanilide	8.1	7.2	0.4	18	0.55	1.2
	Phenacetin	5.8	7.9	0.9	6.4	0.6	1
	Paracetamol	5.4	8.3	0.5	10.8	0.4	0.9
	Salicylamide	60.5	14.4	0.3	48	0.5	1.2
Tricyclic antidepressants	Amitriptyline	34.1	26.7	2.7	9.9	2.1	1.2
	Protriptyline	54.9	23.5	2.6	9	1.75	1.2
	Nortriptyline	30.6	23.7	2.8	8.5	1.75	1.2
	Carbamazepine	16.4	14.3	0.7	20.4	0.8	1.2
	Imipramine	30.4	40.6	0.9	33.8	2.05	1.0
	Desipramine	23.5	34.7	0.7	33.6	1.7	1.0
	Tripramine	29.7	35.2	1.2	24.8	1.8	1.0
	Opipramol	14.2	20.3	1.2	11.8	2.4	1.1
Sulphonamides	Sulphacetamide	8.1	7.1	0.4	17.8	0.6	1.2
	Sulphadiazine	10	7.6	0.6	12.7	0.6	1.1
	Sulphaguanidine	8	8	0.3	26.7	0.6	1.2
	Sulphamerazine	11.4	9.3	0.8	11.6	0.6	1.2
	Sulphathiazol	9.8	7.6	0.3	25.3	0.65	1.2
	Sulphanilamide	7.1	6.4	0.2	32	0.6	1.2
	Sulphapyridine	7.7	9.4	0.5	15.4	0.65	1.1

TABLE I *(continued)*

Group	*Compound*	*MDC (ng on-column)*			*Gain* (ECD)*	*k'*	*Applied potential (V)*
		UV (254 nm) (l = 1)	*UV $\lambda_{max.}$ (l = 0.6)*	*ECD*			
Sex hormones	Diethylstilbestrol	8.3	10.6	1.3	6	0.85	0.7
	Dienoestrol	8.5	–	0.6	14.2	0.9	1.1
	Estrone	126	485	1	126	0.8	1.1
	Ethinyloestradiol	468	113	1.1	102.7	0.7	1.1
	Hexoestrol	214	64.3	0.5	128.6	0.95	1.2
Beta-adrenoceptor blocking agents	Oxprenolol	180	101	1	101	1.95	1.2
	Pindolol	31	25.4	0.7	36.3	1.9	0.9
	Practolol	10.6	14.7	1.4	7.6	1.2	0.9
	Timolol	180	42	2.2	19.1	1.5	1
	Propanolol	90	43	1	43	2.3	1.2
Phenothiazines	Acetophenazine	36	33.4	1	33.4	2.6	1.2
	Chloropromazine	9.5	11	0.5	19	2.35	1.2
	Levomepromazine	7.8	14.5	0.4	19.5	2.4	1.2
	Oxomemazine	75	28.1	0.8	35.1	1.8	1.2
	Perazine	31	–	1.2	25.8	3.8	1.2
	Prochloroperazine	33.3	50	1.2	27.8	3.8	1.2
	Aminopromazine	18	23.4	0.8	22.5	3.3	1.2
	Promazine	10	11	1.1	9.1	2.15	1.2
	Thioproperazine	45	41.8	1.5	27.9	3.1	1.2
	Dimetothiazine	13.4	14.2	0.7	19.1	2.2	1.2
	Thioridazine	13.4	15.7	0.6	22.3	2.6	1.2
Penicillins	Amoxicillin	180	25	1.7	14.7	0.8	1.2
	Meticillin	60	104	1.7	35.3	0.55	1.2
	Oxacillin	50	213	6.4	7.8	0.65	1.2
	Benzylpenicillin	69	72	3.4	20.3	0.4	1.2
	Carbenicillin	35	17	5.2	3.3	0.4	1.2
Diuretics	Triamteren	7.8	9.4	0.6	13	0.8	1.2
	Furosemide	45	18.5	0.8	23.1	0.9	1.2
	Amiloride	22	11.4	0.8	14.3	0.9	1.2
	Hydrochlorothiazide	24.3	14.4	1.1	13.1	1.3	1.2
	Benzthiazide	21.4	9.3	4.8	1.9	0.7	1.2
Alkaloids	Morphine	164	188	1.6	102	2	1.2
	Papaverine	3	5.8	0.7	4.3	1.65	1.2
	Dihydrocodeinone	300	367	1	300	2.3	1.2
	Oxycodone	180	223	0.9	200	2.3	1.2
	Dihydromorphinone	182	204	1.3	140	2.15	1.2
	Heroin	565	251	7.4	34	1.4	1.2
	Apomorphine	18	16.9	0.4	42	1.25	1.2
	Codeine	112	216	0.8	140	1.2	1.2
	Narcotine	60	123	0.6	100	1.5	1.2
	Hydrastinine	25	18	0.8	22.5	2.35	1.2
	Vindesine	125	57	2.4	24	3.85	1.2
	Vincristine	93	50	5	10	4.75	1.2
	Vinblastine	188	88	3.7	24	5.05	1.2
	Desacetylvinblastine	182	83	3.5	24	4.5	1.2

* The factor gain in sensitivity with an amperometric detector.

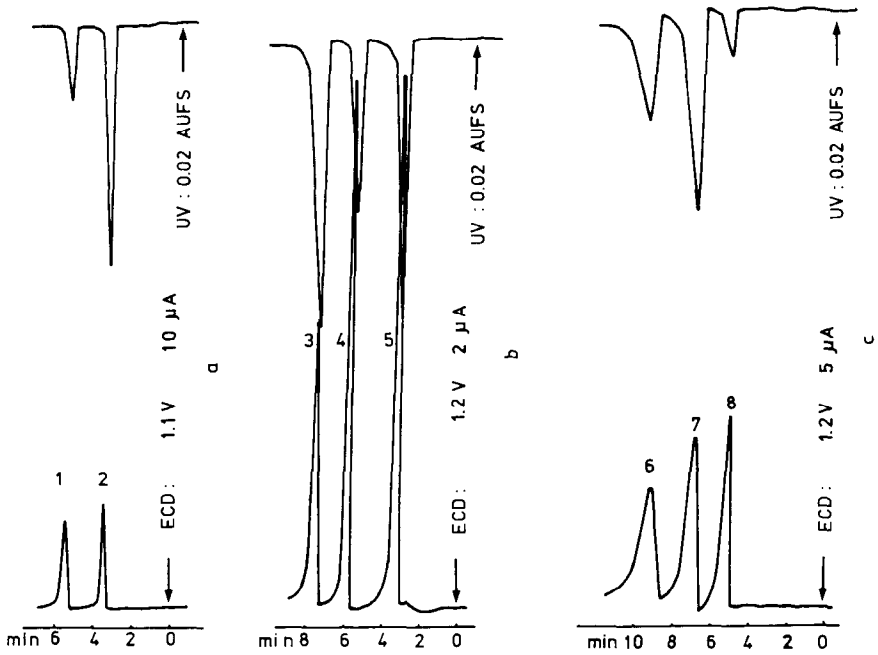

Fig. 1. Chromatograms with simultaneous UV detection at 254 nm and amperometric detection. (a) Local anaesthetics: 1, procaine; 2, benzocaine. (b) Tricyclic antidepressants: 3, opipramol; 4, desipramine; 5, carbamazepine. (c) Phenothiazines: 6, perazine; 7, aminopromazine; 8, oxomemazine. The concentration of all solutes is 1 ppm.

Applications

Local anaesthetics. For the five local anaesthetics investigated, amperometric is more sensitive than UV detection. The applied potential was 1.2 V for all the substances in this group. Nevertheless, procaine, tetracaine and benzocaine can be measured more selectively than amylocaine and lidocaine, as the potential at which a first signal can be measured is 800 mV for procaine, tetracaine and benzocaine and 1 V for amylocaine and lidocaine (Fig. 3). The MDC is lowest for benzocaine and procaine: they possess a primary aromatic amine function. The gain in sensitivity is remarkably high (Table I) for lidocaine, owing to the high MDC-UV rather than to a low MDC-E.

Antipyretics. The advantages of amperometric detection are illustrated by paracetamol: the minimum detectable concentration is a factor of 11 lower compared with UV detection and also the selectivity is high (Fig. 4). The first oxidation is measured at a potential of 500 mV and the limiting current plateau starts at 900 mV. An enhanced sensitivity is also obtained by using an amperometric detector for the other three compounds of this group. Although the selectivity is less pronounced than for paracetamol, the MDC in comparison with paracetamol are lower for acetanilide and salicylamide. Lower sensitivity and selectivity are obtained for phenacetin. Paracetamol contains a phenol function, which is deplaced by an ethyl ether function in the molecular structure of phenacetin.

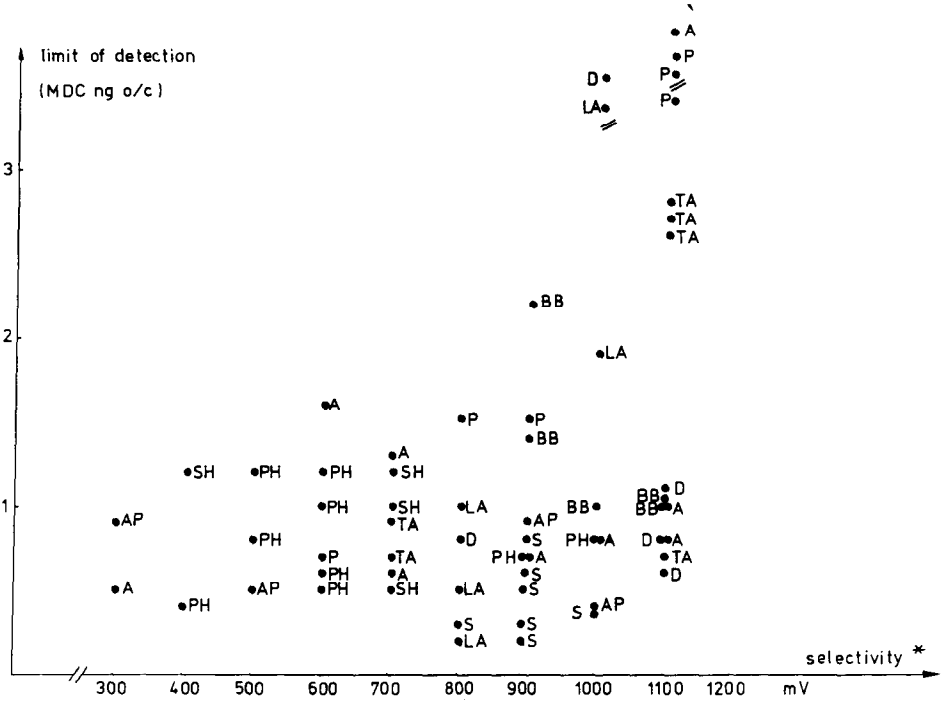

Fig. 2. Electrochemical properties of the drugs investigated. Selectivity* = the potential where the first oxidation signal appears. LA, local anaesthetics; AP, antipyretics; TA, tricyclic antidepressants; S, sulphonamides; SH, sex hormones; BB, β-adrenoceptor blocking agents; PH, phenothiazines; P, penicillins; D, diuretics; A, alkaloids. MDC ng o/c = minimal detectable concentration (ng on-column).

Tricyclic antidepressants. For all compounds, amperometric is more sensitive than UV detection. A great difference in sensitivity and selectivity is noted between the group of imipramines and the group of triptylines (Fig. 5). The former contain an aliphatic and a tertiary amine function, whereas the latter have only an aliphatic amine function. The MDC-E is the same for carbamazepine and desipramine, but the selectivity is much better for desipramine. Opipramol is the only drug in this group that can be monitored at 600 mV; its sensitivity at the applied potential is the same as for trimipramine.

Sulphonamides. The sulphonamides investigated all possess similar electrochemical properties (Fig. 1). The MDC-E are between 0.3 and 0.8 ng on-column and are much lower than the respective MDC-UV values. They all have a primary aromatic amine function. Sulphanilamide is the only compound in this group that contains a free SO_2NH_2 function: its sensitivity is the highest of the group.

Sex hormones. All compounds in this group contain at least one phenol function. The sensitivity is enhanced by the use of an amperometric detector for all the sex hormones investigated, especially for estrone, ethinyloestradiol and hexoestrol, the MDC-E of which are at least 100 times lower than the corresponding MDC-UV values. No MDC-UV value at the absorption maximum is given for dienoestrol as its spectrum, monitored in the mobile phase, showed no real absorption maximum.

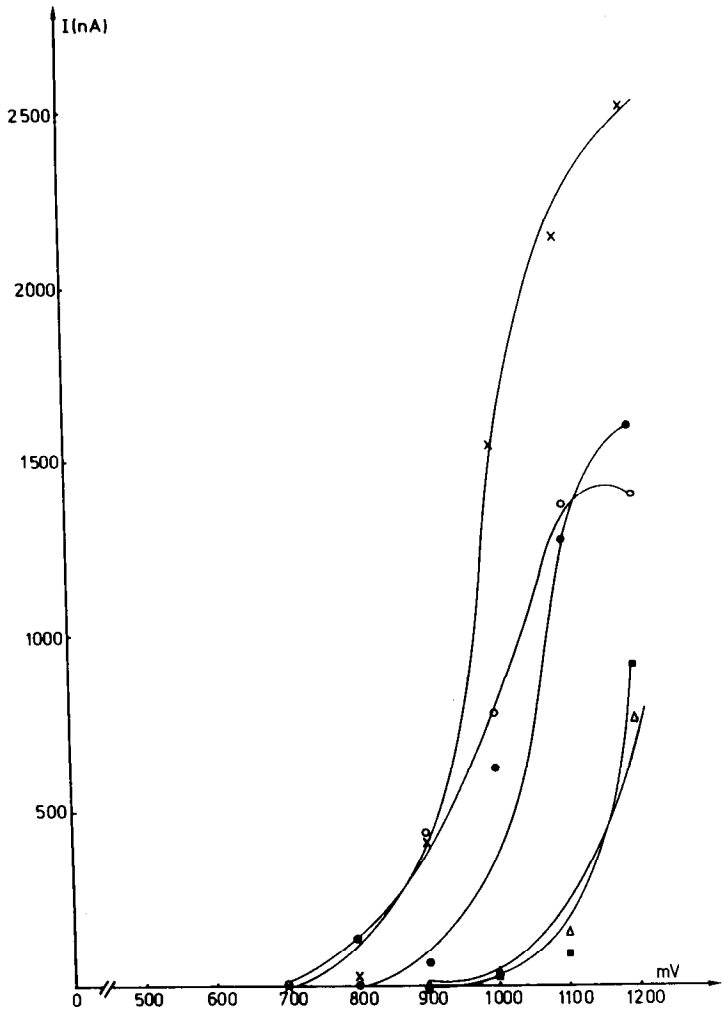

Fig. 3. Voltammogram of local anaesthetics. ○, Tetracaine; ●, procaine; △, amylocaine; ×, benzocaine; ■, lidocaine.

Hexoestrol and dienoestrol contain two phenol functions and their MDC-E values are lower than those for ethinyloestradiol and estrone. The greatest selectivity is obtained for diethylstilbestrol. Although it contains two phenol functions, the sensitivity is half that for the other diphenolic hormones mentioned, probably because the two phenolic nuclei are coupled by one double bond.

Beta-adrenoceptor blocking agents. For all the beta-adrenoceptor blocking agents the sensitivity was considerably enhanced with an amperometric detector. The lowest MDC-E value occurs for pindolol; it also allows more selective detection. For pindolol, practolol and timolol a limiting current plateau is obtained (Fig. 6), whereas this does not occur for the other compounds in this group.

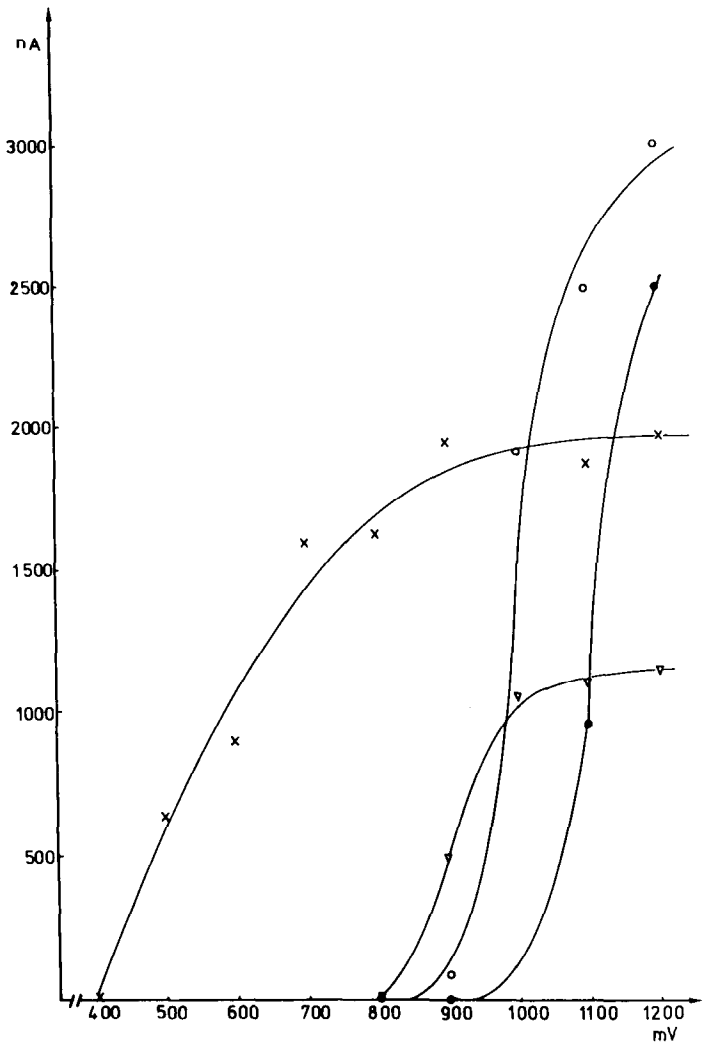

Fig. 4. Voltammogram of antipyretics. ○, Salicylamide; ×, paracetamol; ●, acetanilide; ▽, phenacetin.

Phenothiazines. All MDC-E values are at least nine times lower than the MDC-UV values. For all the compounds except oxomemazine and dimetothiazine a characteristic voltammogram is obtained: a first plateau is reached at 700 mV, but at 1.2 V the sensitivity is considerably increased (Fig. 7). For this reason the MDC-E values were measured at 1.2 V. The lowest MDC-E is obtained for levomepromazine, as it possesses not only the typical phenothiazine structure but also a methyl ether function.

Penicillins. The voltammograms of amoxicillin and meticillin on the one hand and oxacillin, benzylpenicillin and carbenicillin on the other are very different. Amoxicillin and meticillin are more suitable for electrochemical detection as they contain

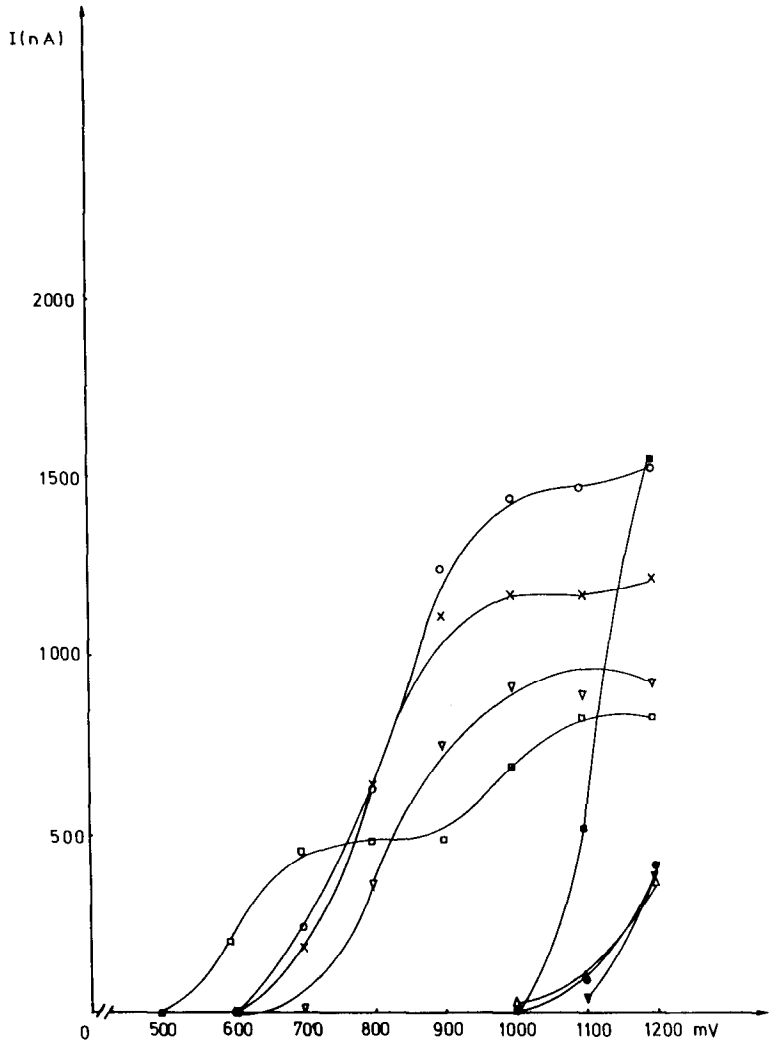

Fig. 5. Voltammogram of tricyclic antidepressants. ○, Desimipramine; ×, imipramine; ▽, trimipramine; ■, carbamazepine; □, opipramol; △, amitriptyline; ●, protriptyline; ▼, nortriptyline.

one phenol and two methyl ether function(s), respectively, whereas the other three penicillins possess only the typical β-lactam structure of penicillins. Although the electrochemical properties are not so favourable for the oxacillin group (Fig. 1), the gain in sensitivity with an amperometric detector is still significant.

Diuretics. Amperometric detection is the most sensitive detection system also for this group of pharmaceuticals. Triamteren and amiloride can be detected in a more sensitive but a less selective way than furosemide and hydrochlorthiazide. The first two contain a primary aromatic amine function, whereas the latter contain a secondary aromatic-amine fuction and an SO_2NH_2 group. Benzthiazide is less suitable for electrochemical detection than the other compounds of this group, owing to

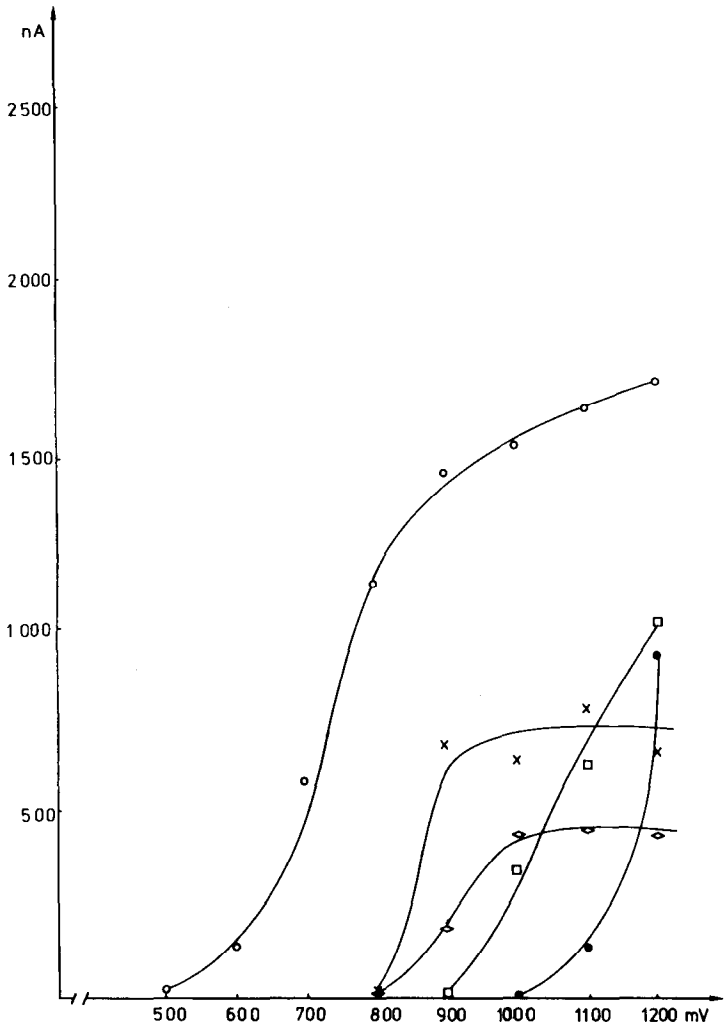

Fig. 6. Voltammogram of beta-adrenoceptor blocking agents. ○, Pindolol; ×, practolol; □, propanolol; ●, oxprenolol; ◇, timolol.

its high MDC-UV value. For these reasons the gain in sensitivity is the lowest for all the drugs investigated.

Alkaloids. The MDC-E values are significantly lower than the respective MDC-UV values also for all compounds of this group, this is certainly the case for morphine, dihydrocodeinone, oxycodone, dihydromorphinone, codeine and narcotine. The highest selectivity and the lowest MDC-E values are obtained for apomorphine, a diphenol. The sensitivity and the selectivity of dihydromorphinone and morphine, both possessing one phenol function, are less than those for apomorphine.

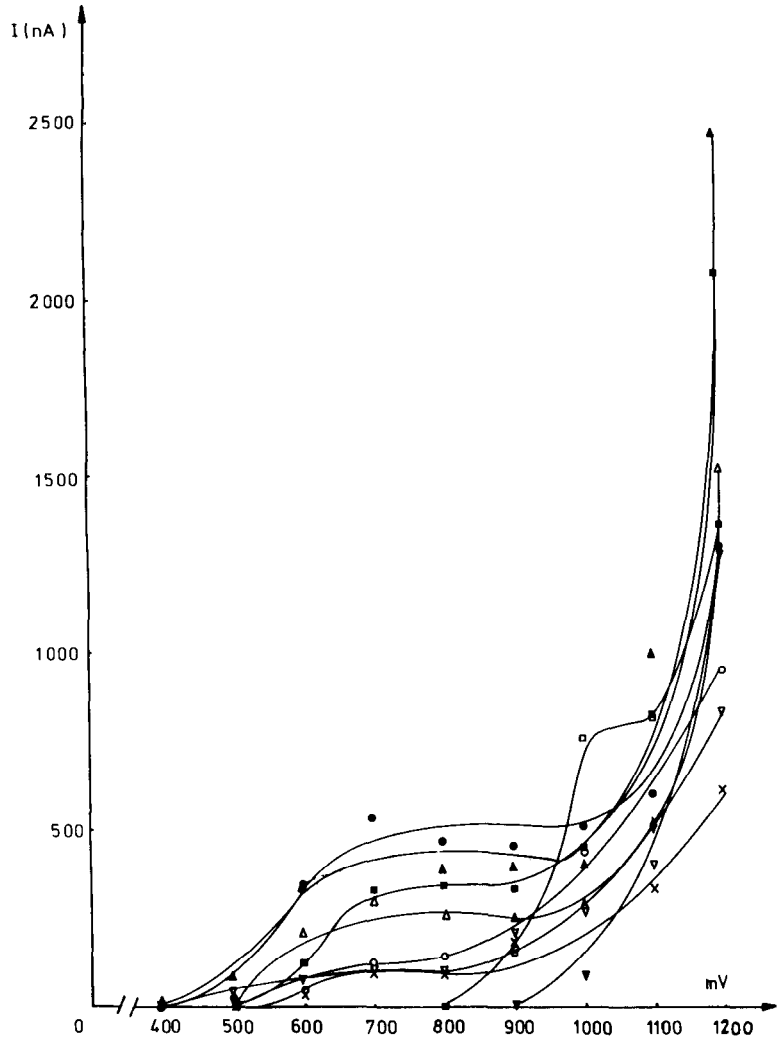

Fig. 7. Voltammogram of phenothiazines. ×, Prochlorperazine; ▽, perazine; ○, acetophenazine; ▼, oxomemazine; ■, chlorpromazine; □, dimetothiazine; △, thioridazine; ●, aminopromazine; ▲, levomepromazine.

CONCLUSIONS

The range of application of amperometric detection in pharmaceutical analysis is very broad. Compounds containing a phenol, a primary or secondary aromatic amine, an aromatic alkyl ether- or a thiol[58,59] function are detectable by amperometric detection. Even when they possess apolar properties, such as some alkaloids, phenothiazines or tricyclic antidepressants, they can be chromatographed in a reversed-phase system, using a buffer system of acidic pH and a relatively large amount

of organic modifier as the mobile phase. Normal-phase elution can be made compatible with electrochemical detection[60,61], but this is more complex.

Amperometric detection is systematically more sensitive than UV detection. In 57 cases out of 72 the gain is at least one order of magnitude (*i.e.*, a factor of 10 or more) and in 11 cases it is even two orders of magnitude higher. The median limit of detection is 1.0 ng on-column and ranges from 0.2 ng (benzocaine, sulphanilamide) to 7.4 ng (heroin). Of course, the limit of detection as determined here depends on the k' value. Therefore, all limit of detections were extrapolated to $k' = 1$. The substances with the lowest MDC-E are now benzocaine, sulphonamide, levomepromazine and apomorphine and the median sensitivity is 0.8 ng on-column. The utility of the electrochemical detector in our expert system approach is mainly as an alternative to UV detection and it is therefore interesting that the median gain is 22.5 and ranges from 1.9 (benzthiazide) to 300 (dihydrocodeinone). Of course, these numbers relate to the particular detection and chromatographic systems used by us, but it should be easy to recalculate them for other similar systems. The low MDC-E value is most pronounced for primary aromatic amine functions and the selectivity for phenol functions. Diphenolic compounds are best suited for electrochemical detection as the selectivity is high and the limit of detection is low. As a general conclusion, we may say that amperometric detection should be investigated by the expert system as a possible detection system in pharmaceutical and certainly in biomedical analyses, because of its relatively broad application range and its improved sensitivity.

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