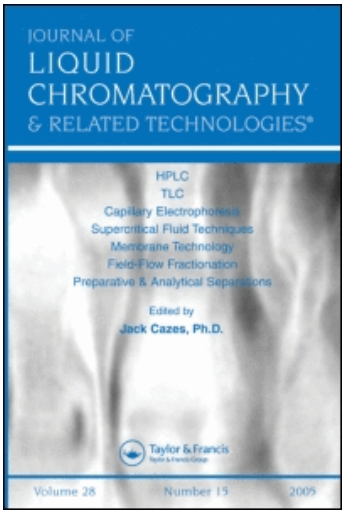

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Musso, N. R. , Vergassola, C. , Pende, A. and Lotti, G.(1990) 'HPLC with Electrochemical Detection of Catecholamines in Human Plasma. A Mini-Review', Journal of Liquid Chromatography & Related Technologies, 13: 6, 1075 — 1090
To link to this Article: DOI: 10.1080/01483919008049235
URL: http://dx.doi.org/10.1080/01483919008049235

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(6), 1075-1090 (1990)

Review

HPLC WITH ELECTROCHEMICAL DETECTION OF CATECHOLAMINES IN HUMAN PLASMA. A MINI-REVIEW

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Introduction

Since 1970s HPLC received growing attention among catecholamine (CA) assay methods. Earlier methods such as colorimetric (1) or fluorimetric (2) nowadays seem obsolete. Radiotracer methods are usually effective for the determination of norepinephrine (NE) and epinephrine (Epi) in human plasma, although the high cost per sample, the poor sensitivity regarding plasma dopamine (DA), the involvement of radiolabelled compounds and the te-

dious and time-consuming assay procedures contributed to the increasing research into different areas for CA assay. Nevertheless radiotracer methods, and primarily radioenzymatic single isotope assay, greatly contributed to the knowledge of the sympathomedullary function.

Gas chromatography with mass spectrometry (GC-MS) for determination of CA, undoubtedly represents a great improvement in sensitivity and selectivity respect to the aforementioned methods. However, owing to the very high cost of GC-MS equipments, this is not an expanding field.

Among the number of various HPLC techniques for CA assay, those employing electrochemical detection (ECD) emerged . In the last decade ECD became the detection of choice when HPLC assay of CA is attempted, owing to its sensitivity and specificity and to the outstanding technical development in the manufactoring of these equipments (3). Turning to chromatographic separations, the most popular and effective ones seem to be the ion-exchange and the ion-pair reversed-phase chromatography, because of their straightforward separations (4). These HPLC

techniques require similar extraction and cleanup procedures for plasma samples, generally based on alumina - batch methods (5) . These two HPLC techniques, coupled with ECD, have gained a widespread use and are the most promising procedures in the field of CA assay. In the present paper we will analyze the results obtained by different Authors employing these last techniques. This review is addressed to the clinical laboratory practice, and is not in competition with thorough reviews in the field of the sympathomedullary assessment.

Chromatographic Separation of Catecholamines

Ion-exchange columns were used for quantitative analysis of the basic CA, meaning that cation-exchange resins were employed. The separation process is attained on the basis of the retention of the protonated aminogroup of the CA. This separation procedure was lacking of interest in the last years. Earliest applications of ion -ex chromatography were conducted by the use of relatively large pellicular packing materials, showing broadening and tailing of the peaks, and

poor CA detectability (6). More recently, some Authors showed novel properties of smaller packing materials and favourable applications of ion-ex chromatography in this field (7,8).

Ion-pair reversed-phase chromatography is the dominating procedure today in the CA separation. This HPLC technique (otherwise called 'soap' chromatography) is claimed to display sharp chromatograms, the peaks being usually narrow and symmetrical, and to favour the enhanced sensitivity of ECD. Moreover the chromatographic separation may be easily manipulated by very low variations in the pH of the mobile phase (generally between 3.00 and 3.50). This procedure can change dramatically the elution time of interfering compounds (such as DOPA or DOPAC), giving clean elution profiles. Many Authors showed good or improved CA assay by the use of soap chromatography, nevertheless the use of 25-30 cm long columns (9,10) and radial pack cartridges (11) is effective but slow; other methods are insensitive for DA basal circulating levels (12). More recently, the use of short (7.5 cm x 4.6 mm id) reversed-phase columns, packed with 3μm particle size materials (13,14), was shown to

have great selectivity and rapidity (separation = 5 min), and to favour the excellent sensitivity of ECD. Moreover plasma DA seems to be constantly detectable in human plasma in basal conditions (14). Although 3μm packing materials are claimed to give technical difficulties and easier clogging, in our experience these problems may be avoided by careful filtration (0.2μm) of both mobile phase and samples, and by the use of mobile phase filters and appropriate precolumns. In addition, these procedures preserve from clogging the very small bore (0.007" id) tubes plumbed in these systems.

Detection

HPLC with ECD has been proven as a useful tool in the study of the sympathomedullary physiology. Further, this procedure has gained a widespread use owing to its excellent sensitivity to low amounts of CA (in the picogram range). ECD is based on the principle that such compounds (so-called electro-active) may oxidize at a certain potential. This oxidation liberates electrons and creates a

measurable current. In addition, CA are compounds which are reversibly oxidized to quinones. These may be, in turn, re-reduced to CA.

The most widely used electrochemical detectors are the amperometric ones from Bioanalytical Systems (BAS, West Lafayette, IN) and the coulometric ones from ESA (Bedford, MA).

Amperometric detectors utilize thin-layer flow cells and carbon paste or glassy carbon working electrodes vs Ag/AgCl reference electrodes. These electrodes showed an excellent sensitivity (in the picogram range) to catecholic compounds (13,15), although sensitivity and performances of these detectors depend on the electrode surface being clean and absolutely smooth (16). Further, stability of carbon paste electrodes is unpredictable and a decrease in performances may occur within minutes to days (16). In addition it should be remembered that a number of pitfalls, such as noise problems subsequent to static electricity (the same technician must be grounded), electromagnetic interferences, radiofrequency interferences, electrical disturbances in power lines (17), can obscure the results. Then,

maintenance and troubleshooting times may be frustrating. Nevertheless, performances of amperometric detectors in experienced hands are satisfactory and good results were shown in small volumes of plasma (13).

Coulometric detectors consist in one or more flow-through electrodes of fully-porous graphite. These electrodes can react with 70-99% of the electroactive components of the analyte. The principal advantages over amperometry are: a) a greatly reduced maintenance time (i.e. a simple flush by 6 Mol/ L nitric acid of the cells every 6 months); b) the possibility and the reliability of making series of more electrodes (baseline noise being reduced by irreversible oxidation of the interfering compounds); c) the better sensitivity; d) the quantitative reaction of the compounds of interest (about 90% respect to 5% in amperometry).

Recently some Authors showed the great performances of short series of coulometric detectors in the assay of tissue CA (18) and plasma DA (14). Also recently, series up to 15-16 electrodes were shown to have great selectivity in CA assay by injecting in the system the plasma

sample after simple filtration, so bypassing the extraction procedure (19). The principal advantage in the use of series of electrodes is to select an oxidation - reduction chain on the voltammogram basis. This procedure, by reaction with electrodes of all electroactive components of the sample, allows increased sensitivity, owing to the drastic decrease of the baseline noise (18,19). In addition specificity is also increased by this procedure. The composition of the mobile phase can influence the sensitivity of ECD. The use of high pure

TABLE 1

Mean (and SD) Values of Basal Levels of Venous CA in Normal Humans, Obtained by Radioenzymatic (RE) or HPLC ECD (LCEC) Assays.

Ref.	year	n	Method	NE(SD)	Epi(SD)	DA(SD)
(6)	1978	6	LCEC	519(174)	40(45)	0 - 23
(20)	1980	10	RE	196(53)	36(13)	62(16)
(10)	1981	5	LCEC	292(108)	81(60)	29(6)
(21)	1981	10	LCEC	304(19)	72(23)	- -
(22)	1981	13	RE	286(159)	49(34)	- -
(23)	1985	11	RE	250(86)	33(15)	39(22)
(24)	1986	70	RE	238(92)	28(33)	31(42)

Values are in pg/mL. n: number of subjects.

chemicals is needed in HPLC; when ECD is employed chemicals must be of the best quality. It should be borne in mind that no suppliers offer 'electrochemical pure' reagents; the liquid chromatographer must test a number of reagents in order to find the adequate one. As a general finding, low amounts of EDTA (max 1mMol/L) can help to enhance the sensitivity of the detection, in part suppressing the baseline noise.

Levels of Catecholamines in Human Plasma

Considering plasma basal values of CA in man, we compared data obtained by different methods from the analysis of peripheral venous blood (Table 1).

Using an HPLC ECD method developed in our laboratory (14) we determined in the basal state the plasma CA levels in 64 normotensive subjects aged 20-58, in supine position 30 min after the insertion of a venous catheter in a forearm vein. Results were: NE 150.4 (51.3); Epi 48.4 (16.2); DA 22.7 (12.2). Values are mean (and SD), expressed as pg/mL. In particular plasma DA was detectable in

each subject, varying from 8 to 80 pg/mL, and showing a Poisson distribution. Results obtained using RE assay generally agreed with those obtained using HPLC ECD, the common problem being the frequent undetectability of the basal levels of free DA. In front of the impressive number of technical papers describing analytical methods for NE, Epi and DA determination, clinical reports concerning CA are often limited to NE and Epi, because DA usually is undetectable in human venous plasma. Coupling a 3-electrodes series with 3μm particle size reversed-phase short column, we obtained a better sensitivity (i.e. an enhancement of the detector response) for DA respect to previous methods. This allowed us to avoid undetectable values in each subject studied. Moreover the sensitivity of this method allows to detect variations in plasma DA as low as 4 pg/mL (detection limits; signal to noise ratio 3:1).

Research and Routine Applications

Radiotracer methods allow few, high cost determinations per working day (about 8-12 samples

per day; about 50$ each), and are not suitable for routine analyses. These techniques are generally tedious and capricious, and their application in some pathophysiologic fields is an intriguing question (25,26).

HPLC ECD equipments are expensive, but determinations are not. The chromatographic analysis of plasma CA by amperometry is a useful tool in research fields, but to a lesser extent in routine determinations. The principal problems arising from the amperometric analysis of plasma CA are the high and unpredictable maintenance rate of the electrodes and the poor sensitivity regarding basal levels of circulating DA. On the contrary, HPLC ECD with coulometric detection is an affordable technique both in research and routine analysis fields, owing to its excellent sensitivity to low concentrations of CA (13,14,18) and to its very low (i.e. every 6 months) maintenance rate. These methods allow non-stop function of the apparatus for a large number of determinations to be done. The cost per each determination may be as low as 5$ (14), and the number of determinations per day as high as 70 (14).

Conclusions

Radiotracer methods for CA assay are still in use but not reliable for research and routine analysis. These techniques are tedious, money- and time - consuming.

HPLC ECD techniques either by amperometry or coulometry have gained a widespread use in CA assay. Among these methods, reversed-phase ion-pair chromatography with coulometric detection probably allows the best results. Further, the use of short, 3μm particle size columns enhances the sensitivity and selectivity of ECD. This procedure makes the quantitation of plasma CA reliable in the laboratory routine and in the clinical research.

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Received: November 27, 1989
Accepted: January 22, 1990