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Electrochemical methods for monitoring of environmental carcinogens

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Abstract The use of modern electroanalytical techniques, namely differential pulse polarography, differential pulse voltammetry on hanging mercury drop electrode or carbon paste electrode, adsorptive stripping voltammetry and high performance liquid chromatography with electrochemical detection for the determination of trace amounts of carcinogenic N-nitroso compounds, azo compounds, heterocyclic compounds, nitrated polycyclic aromatic hydrocarbons and aromatic and heterocyclic amines is discussed. Scope and limitations of these methods are described and some practical applications based on their combination with liquid-liquid or solid phase extraction are given.

Abbreviations AdSV, adsorptive stripping voltammetry; CPE, carbon paste electrode; DME, classical dropping mercury electrode; DPP, differential pulse polarography; DPV, differential pulse voltammetry; eaGCE, electrochemically activated glassy carbon electrode; GCE, glassy carbon electrode; HMDE, hanging mercury drop electrode; HPLC-ED, high performance liquid chromatography with electrochemical detection; LLE, liquid-liquid extraction; LOD, limit of determination; NPAH, nitrated polycyclic aromatic hydrocarbons; SMDE, static mercury drop electrode; SPE, solid phase extraction; TLC, thin layer chromatography

1 Introduction

Even extremely low concentrations of environmental carcinogens can increase the occurrence of various forms of cancer [1]. Therefore, large scale monitoring of environmental carcinogens is becoming more and more important [2]. For the volatile carcinogens, the GC is obviously the method of choice with well-known sensitivity and selectivity. UV photometry with LOD from 10^{-6} to 10^{-7} mol/L is usually not selective enough for more complex matrices. HPLC with UV (LOD ~ 10^{-6} mol/L), fluorimetric (LOD ~ 10^{-9} mol/L), chemiluminescence (LOD ~ 10^{-10} mol/L) or MS detection (LOD ~ 10^{-11} mol/L) are more frequently used. Preparation of samples for analysis depends on the nature of the sample. Owing to the complexity of matrices in which chemical carcinogens are sought, sample preparation is usually a multistep operation, involving extraction, filtration, fractionation, drying of extracts, redissolution and other steps. A continuously updated list of substances carcinogenic to humans, substances probably carcinogenic to humans and substances possibly carcinogenic to humans can be found on web pages of the International Agency for Research on Cancer [3]. Analytical measurement procedures should have a critical role in molecular epidemiology and exposure regulation, as well as in environmental monitoring. We believe that modern electroanalytical methods can play a useful role in this field [4, 5] because they are extremely sensitive, exhibit extraordinary broad linear dynamic range and are inexpensive from the point of view of both investment and running costs. Moreover, they present an independent alternative which can be used for the confirmation of the results obtained by so far prevalent spectrometric or chromatographic techniques. In some cases, there is a relationship between polarographic and/or voltammetric behavior and genotoxic properties of organic compounds [6–10] and the knowledge of the mechanism of their electrode reactions can give us a useful clue in elucidation of the mechanism of their interaction with living cells and their fate in the environment. For the above reasons, we have systematically investigated the polarographic and voltammetric behavior of several groups of chemical carcinogens using differential pulse polarography (DPP) on the classical dropping mercury electrode (DME) or the static mercury drop electrode (SMDE), differential pulse voltammetry (DPV) on the hanging mercury drop electrode (HMDE), the glassy carbon electrode (GCE) or the carbon paste electrode (CPE), adsorptive stripping voltammetry (AdSV) and high performance liquid chromatography with electrochemical detection

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(HPLC-ED). Scope and limitations of methods for the determination of trace amounts of carcinogenic N-nitroso compounds, azo compounds, heterocyclic compounds, nitrated polycyclic aromatic hydrocarbons and aromatic and heterocyclic amines developed in our UNESCO Laboratory of Environmental Electrochemistry at Charles University, Prague and in other laboratories will be discussed and some practical applications based on their combination with liquid-liquid (LLE) or solid phase extraction (SPE) will be given.

2 Polarography and voltammetry on mercury electrodes

Many important environmental carcinogens are polarographically active (see Table 1) and thus amenable to the determination based on their reduction on a suitable type of mercury electrode. DPP at DME is more suitable for the determination in more complicated matrices because the slope of the calibration curve is less dependent on the presence of surface active substances and some other possible interferences. From this point of view, DME is still the best electrode available because of the periodical renewal of its surface which minimizes problems with passivation and electrode fouling. However, the limit of determination (LOD) is usually around 1×10^{-7} mol/L, which is not enough for the most demanding applications. AdSV on HMDE is much more sensitive with a typical LOD between 10^{-9} and 10^{-10} mol/L. Unfortunately, it is more prone to complications connected with the fact that the electrode surface is not renewed during the measurement.

N-nitroso compounds, which are among the most commonly occurring chemical carcinogens, are polarographically reduced in alkaline medium with an exchange of two electrons and in acidic medium with the exchange of four electrons. It means that the limiting diffusion current is thus roughly twice higher in acidic than in alkaline medium. The polarographic determination of these compounds is thus carried out usually at low pH, the limit of determination being around 10^{-5} mol/L for DC polarography and 10^{-7} mol/L for DP polarography. Numerous references on the polarographic determination of various N-nitroso compounds can be found in review [11] or original paper [12]. Carcinogenic N-nitrosomorpholine that is produced in Diesel motor combustion can be determined by square wave polarography [13]. The danger of false positive results when determining trace amounts of these substances in food [14], spirits [15], and beer [16, 17] can easily be eliminated by UV irradiation resulting in the disappearance of the DPP peak of photo labile N-nitroso compounds. Unfortunately, N-nitroso compounds do not adsorb on HMDE and thus cannot be determined using AdSV.

Azo compounds are another group of suspected chemical carcinogens which are easily polarographically reducible [18, 19]. Genotoxic derivatives of N,N-dimethyl-4-aminoazobenzene can be determined using DC polarography with LOD around 10^{-5} mol/L [20, 21] while DPV or AdSV gives much lower LOD (see Table 1). The polarographic behavior of derivatives with polarographically inactive constituents depends on pH because azo group can be reduced to hydrazo or amino groups. The polarographic behavior of N,N-dimethyl- 4-amino-3′-nitroazobenzene is even more complicated because of the presence of a nitro group which can be reduced with the exchange of 2, 4 or 6 electrons to -NO, -NHOH or -NH_2 group in dependence on the pH [22]. Similarly, the mechanism of reduction of N,N-dimethyl-4-amino- 4′-aminoazobenzene is affected by the mutual interaction of azo and amino group [23]. The selectivity of these methods enables the direct determination of N,N-dimethyl- 4-amino-

Table 1 Selected examples of polarographic and voltammetric determination of chemical carcinogens at mercury electrodes

Substance	Technique	Medium	LOD, mol/L	Ref.
N – Nitrosocompounds				
N-Nitroso-N-methylanilines	DPV/HMDE	BR pH 3.5	1×10^{-7}	41
N,N′-Dinitrosopiperazine	DPV/HMDE	BR pH 2.4	7×10^{-8}	37
Azo compounds				
3′-Halogen-N,N-dimethyl-4-aminoazobenzene	AdSV/HMDE	BR-MeOH (1 : 9) pH 2.8	2×10^{-9}	97
4′-Nitro-N,N-dimethyl-4-aminoazobenzene	AdSV/HMDE	BR-MeOH (4 : 6) pH 8.4	2×10^{-9}	22
2′-Carboxy-N,N-dimethyl-4-aminoazobenzene	AdSV/HMDE	100 times diluted BR pH 4	1×10^{-9}	25
4′-Sulfo-N,N-dimethyl- 4-aminoazobenzene	AdSV/HMDE	100 times diluted BR pH 9	3×10^{-10}	26
Heterocyclic compounds				
Acridine	AdSV/HMDE	BR-MeOH (1 : 1) pH 6	2×10^{-8}	98
Benz[c]acridine	AdSV/HMDE	BR-MeOH (1 : 1) pH 4.1	5×10^{-9}	98
Dibenz[a,h]acridine	AdSV/HMDE	BR-MeOH (1 : 9) pH 4.8	2×10^{-8}	98
Nitrated polycyclic aromatic hydrocarbons				
4-Nitrobiphenyl	AdSV/HMDE	BR-MeOH (1 : 1) pH 4.8	2×10^{-9}	99
9-Nitroanthracene	AdSV/HMDE	BR-MeOH (9 : 1) pH 7.1	2×10^{-9}	36, 40
1-Nitropyrene	AdSV/HMDE	BR-MeOH (99 : 1), pH 2	1×10^{-9}	100

BR – Britton-Robinson buffer

azobenzene in blood plasma [24] or analysis of a mixture of azobenzene, N,N-dimethyl-4-aminoazobenzene and N,N-dimethyl-4-amino- 4′-aminoazobenzene by DPP. Moreover, this selectivity can be increased by a preliminary separation using extraction by diethylether [25] or n-butanol [26] or by thin layer chromatography (TLC) [27]. The strong adsorption of these substances which is exploited in AdSV can be modeled mathematically [28]. More details can be found in reviews [4, 11].

Heteroaromatic compounds with a π-electron deficit (such as carcinogenic derivatives of acridine) are polarographically reducible and can be determined using modern polarographic and voltammetric techniques in aqueous-methanolic solutions (see Table 1). However, their half-wave potentials are rather negative which increases the LOD. The polarographic behavior of acridine and its derivatives in DMSO [29] and DMF [30, 31] has been examined in relation to their carcinogenity. The polarographic behavior of acridine can be influenced by the formation of an inclusion complex with β-cyclodextrin [32]. Further references on the polarographic behavior of heteroaromatic compounds can be found in [11, 98].

Nitrated polycyclic aromatic hydrocarbons (NPAH) are especially well suited for polarographic or voltammetric determination on mercury electrodes, because they contain easily reducible nitro groups [33, 34] and an aromatic system enabling their adsorption on mercury electrodes and thus their determination using AdSV (see Table 1 and reviews [35, 36]).

The selectivity of polarographic and voltammetric methods on mercury electrodes can be increased and their LOD decreased by preliminary separation and preconcentration of the analyte by LLE [25, 26, 36–38] or SPE [36, 40]. Mixtures of N-nitroso-N-methylanilines [39] or N,N-dimethyl-4-aminoazobenzene derivatives [23, 27] were analyzed by DPP at DME or DPV at HMDE after their separation by TLC.

3 Voltammetry on solid and paste electrodes

These electrodes should be applied mostly for the determination of polarographically inactive carcinogens because for polarographically active substances mercury electrodes would give lower detection limits. Moreover, solid electrodes usually require mechanical, thermal, electrochemical or chemical pre-treatment for sensitive, reproducible and reliable measurement [40]. Nevertheless, in many cases they can be successfully applied for the determination of trace amounts of chemical carcinogens.

A cellulose acetate butyrate polymer modified gold electrode can be used for the determination of N-nitroso-N-butyl-N-propylamine over a 10^{-10} to 10^{-6} mol/L concentration range via AdSV [41] probably due to the anionic butyrate groups within the modifying polymer facilitating nitrosamine sorption. Cathodic stripping voltammetry on Nafion coated glassy carbon electrodes based on preconcentration through chemical reaction can be used for the determination of 4-nitroso-N,N-diethylaniline at nanomolar concentrations. Preconcentration is based on subsequent reduction of 4-nitroso-N,N-diethylaniline to N,N-diethyl-p-phenylene-diamine which then reacts with 4-nitroso-N,N-diethylaniline to form 4,4′-(N,N-diethylamino)azobenzene which can be accumulated and subsequently reduced [42] giving LOD around 3×10^{-9} mol/L. A comparable stripping method based on the direct reduction of 4-nitroso-N,N-diethylaniline has an LOD around 1×10^{-7} mol/L. It is obvious that in these cases the application of solid electrodes is fully justifiable.

Anodic oxidation of N,N-dimethyl-4-aminoazobenzene at a glassy carbon electrode can be used for its determination [43] with an LOD around 1×10^{-5} mol/L, i.e. much higher than at mercury electrodes. One advantage of solid electrodes in this field is their applicability in strongly acidic media, where the half-wave potential of the azo group reduction is too positive for mercury electrodes to be used and where anodic voltammetry can be used for monitoring the efficiency of the destruction of carcinogenic azobenzene derivatives. Another possible advantage is the chance to determine both azobenzene derivatives and corresponding amines formed by their reductive splitting using single GCE. However, mercury electrodes should be preferred for azobenzene derivatives because of higher reproducibility and lower LOD.

The same is valid for heteroaromatic compounds. Possible exception is the investigation of voltammetric behavior of solid micro particles of acridine and other insoluble substances immobilized on the surface of graphite electrode [44].

An interesting application of solid electrodes for the determination of nitrated polycyclic aromatic hydrocarbons is the use of electrochemically activated glassy carbon electrodes (eaGCE) for the determination of 1-nitropyrene with LOD around 2×10^{-8} mol/L [45]. Electrochemical pre-treatment was performed by anodizing the GCE at +0.2 V for 3 min followed by cathodizing at –0.6 V for 2 min or cycling until the stable cyclic voltammogram was obtained. The preconcentration of 1-nitropyrene was performed by immersing the dried eaGCE into the deaerated methanolic solution of the analyte for 10 min and rinsing the electrode with deionized water. Cyclic voltammogram was then recorded at a scan rate of 100 mV/s. The method, which is very simple and fast when compared with HPLC, was applied for the determination of 1-nitropyrene in air particulate.

Aromatic amino compounds, which form another important group of chemical carcinogens, are easily anodically oxidizable at solid or paste electrodes (see review [46]). This fact was exploited for the determination of 4-aminobiphenyl [47] or benzidine derivatives [48] on GCE or 2-, 3- and 4-aminobiphenyl [49], 2-aminofluorene and 2,7-diaminofluorene [36] and some other carcinogenic aromatic amines [50] on CPE. CPE exhibit some unique properties, such as low background current, favorable anodic potential range, extraction capabilities, and easily renewable electrode surface [51] which makes them particularly suited for the determination of carcinogenic aromatic amines. Polymer-coated solid electrodes

[52] are another promising type of electrodes for this purpose which can be demonstrated on the determination of 4-nitroso-N,N-diethylaniline with detection limit of 3×10^{-9} mol/L [42]. Adsorptive accumulation can enable the determination of aromatic amines with LOD around 1×10^{-7} mol/L [53]. Aromatic amines are strongly adsorbed on electrochemically pre-treated GCE [54]. Nevertheless, anodic voltammetry at solid electrodes is more frequently used for research purposes than for routine analytical determinations because of frequent problems with reproducibility. These methods obviously perform much better in the hands of skilled electrochemists than in the hands of analytical chemists without special training in electrochemistry. The pretreatment of the working electrode can be the main problem for non-electrochemists. The passivation of the electrode can easily decrease the LOD by one or two orders of magnitude.

4 HPLC with electrochemical detection

HPLC-ED has always been and will be a more specialized approach as compared with UV or fluorescence detection. However, in certain cases it can give higher sensitivity or selectivity than spectrometric detection techniques. A thorough survey of HPLC-ED applications can be found in monographs [55, 56]. A list of more than one thousand electroactive compounds amenable to HPLC-ED can be found on Internet [57].

HPLC-ED of N-nitroso-N-methylaniline derivatives with DC voltammetry on HMDE gives LOD around 10^{-5} mol/L, anodic voltammetry on a glassy carbon fiber array detector around 10^{-7} mol/L and indirect anodic voltammetric detection after photolytic denitrosation of the analytes yielding corresponding aromatic amines around 10^{-7} mol/L [58]. Acetonitrile-0.01 M KH_2PO_4 (7 + 3) mixture with pH 5.6 was used as mobile phase.

HPLC-ED with 0.01 M NaH_2PO_4-methanol (9 + 1) mixture with pH 4.5 as mobile phase was used for the analysis of mixtures of 13 derivatives of N,N-dimethyl-4-aminoazobenzene [59], the LOD being around 10^{-5} mol/L for cathodic DC polarographic detection on HMDE and around 10^{-7} mol/L for anodic DC voltammetric detection on a glassy carbon fiber array detector.

Electrochemical cells used for HPLC-ED determination of NPAHs are of thin-layer [60–62] or wall-jet [63] type with a glassy carbon, a gold/mercury or a porous graphite working electrode [64]. They are mostly operated in reductive amperometric mode with the working potentials typically between –0.50 V and –0.65 V. A large-area porous graphite working electrode [65] allowed not only the determination of NPAHs in reductive amperometric mode, but the high degree of electrochemical conversion to aromatic amines can also be used in subsequent fluorescence detection. A flow-through, or porous coulometric detector, which has the column eluent actually passing through the porous graphite working electrode can be very useful for the determination of trace amounts of NPAH. The surface area of the working electrode is large, resulting in 100% of analyte reacting, and no signal is wasted resulting in a twenty-fold improvement in signal over other amperometric detectors [62]. Another possibility is to use post column electrochemical reduction of NPAHs to amino derivatives followed by fluorescence detection [66, 67]. The development of the coulometric electrode array system with 16 coulometric porous electrodes allows the simultaneous use of 16 different potentials [68] which can give information about peak purity and peak identity. An attempt [69] to combine HPLC with AdSV on HMDE for the determination of 2-nitrofluorene and 2,7-dinitrofluorene has led to relatively high LOD (around 2×10^{-7} mol/L). HPLC of NPAH with polarographic detection on HMDE gives according to our experience LOD around 1×10^{-5} mol/L, i.e. too high for environmental applications. More information on HPLC-ED of NPAH can be found in a review [70].

Approximate electrode potential for HPLC-ED determination of aromatic amines and benzidines is +1.0 V and +0.6 V vs SCE, respectively, the LOD being 10–100 times lower than for UV detection [61]. The technique was applied for environmental samples and body fluids [61]. The easy anodic oxidation of aromatic amines is successfully used in our UNESCO Laboratory of Environmental Electrochemistry for their HPLC-ED determination with commercially available thin-layer detector [71] or coulometric detector with 2 porous graphite electrodes [72]. Very good results were obtained using carbon-fiber detector [73], platinum tubular detector [74] or thin-layer detector with boron-doped diamond working electrode [75]. Diamond film electrodes with poly-crystalline, boron-doped diamond thin film [76] are especially promising because of low and stable background current, broad potential window and very low noise. The frequent use of HPLC-ED in this field can be demonstrated by the determination of 1-aminopyrene [76], 3-aminofluoranthene [77], 1-aminonaphthalene [74], benzidine and dichlorobenzidine [78] and some other carcinogenic aromatic amines [79] and of benzidine derivatives in laboratory wastes [63], in water and soil [80], in urine [81], and in waste water [82]. We believe that HPLC-ED can be used for biological monitoring of the human exposure to NPAHs based on the determination of corresponding metabolites (aromatic amines) in body fluids in a similar way as competitive ELISA [83]. The HLPC-ED determination of 1-hydroxypyrene in urine as a biomarker for exposition to pyrene with LOD around 5×10^{-7} mol/L can serve as an example [84]. Further examples of HPLC-ED of aromatic amines can be found in a review [46]. Two new reagents, 2,5-dihydroxyphenylacetic acid, 2,5-bis-tetrahydropyranyl ether p-nitrophenyl ester and homogentisic gamma-lactone tetrahydropyranyl ether, were used for the chemical derivatization of primary and/or secondary amines to form an electrochemically active product for HPLC-ED. These reagents undergo reaction with the biogenic amines to form a product possessing the hydroquinone moiety, thus allowing for reversible electrochemical detection at mild oxidation potentials. The reactivity of each reagent was demonstrated by using N-ethyl-

benzylamine [85] but it can be expected that other aromatic amines will react similarly. Aromatic amines at μg/L levels in water were determined with cloud point preconcentration followed by HPLC with UV or electrochemical detection [86]. Determination of aromatic amines in environmental water samples by selective SPE and HPLC with amperometric detection gave LOD from 15 ng/L to 440 ng/L [87]. An interesting new approach, amperometric detection using a cobalt wire working electrode after separation by ion-moderated partition chromatography, was so far applied only for aliphatic amines with LOD of the order of nanomoles [88].

A number of mutagenic/carcinogenic heterocyclic amines were determined by HPLC-ED in various kinds of food [89]. The following polar heterocyclic amines were detected: 2-amino-3-methylimidazo[4,5-f]quinoline, 2-amino-3-methylimidazo-[4,5-f]quinoxaline, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline, 2-amino-3,7,8-trimethylimidazo[4,5-f]quinoxaline, and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. The LOD was in most cases around 1 ng/g. The same holds for the following non-polar heterocyclic amines: 2-amino-9H-pyrido[2,3-b]indole, 2-amino-3-methyl-9H-pyrido[2,3-b]indole, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole, and 3-amino-1-methyl-5H-pyrido[4,3-b]indole. Of the co-mutagenic heterocyclic amines, 1-methyl-9H-pyrido[3,4-b]indole and 9H-pyrido[3,4-b]indole were detected. It was shown by an intercomparison study [90] that four heterocyclic aromatic amines (2-amino-3-methylimidazo[4,5-f] quinoline, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline) and 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine) can be determined in cooked food by HPLC-ED in the range of 1–30 ng/g. Heterocyclic aromatic amines (2-amino-1-methyl-imidazo[4,5-f]quinoline, 2-amino-3,8-dimethyl-imidazo-[4,5-f]quinoline, 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline) in fried poultry meat were determined by HPLC-ED after acid-base partition cleanup followed by SPE with LOD around 1 μg/kg [91].

According to our experience, HPLC-ED combines separation power of chromatography with high sensitivity of electroanalytical techniques, which makes it extremely useful for the determination of trace amounts of chemical carcinogens in complex matrices. However, it is necessary to keep in mind that only electrically conductive mobile phases can be used (which requires the addition of the base electrolyte), that gradient elution is usually not compatible with electrochemical detection and frequent preconditioning of the working electrode is required. The response of the electrochemical detector is usually more dependent on the matrix than is the case with UV photometric detector. Thus, the standard addition method should be preferred.

5 Some novel electroanalytical approaches

Attention was also paid to the determination of both electrochemically active and electrochemically inactive chemical carcinogens based on their interaction with electrodes modified by DNA [92, 93]. This very promising new approach initiated by Paleček and co-workers [94] is based on the voltammetric or chronopotentiometric detection of the changes of the redox signal of DNA immobilized on the surface of the working electrode resulting from its interaction with the analyte. This approach is very promising, especially for chemical carcinogens, in which case this interaction is especially strong. Intercalative collection of carcinogenic aromatic amines onto the double-stranded DNA immobilized on the surface of CPE gives nanomolar detection limits after 10 min accumulation [98]. Moreover, this type of device holds great promise for elucidating molecular interactions of various type of carcinogens with DNA.

Another interesting possibility is the application of so called „biologically modified carbon paste electrodes“ [95] based on the incorporation of biological entities within carbon pastes. Modification of electrode surfaces involves a great number of methods of surface activation and immobilization of selective chemical and/or biochemical systems on the electrode surface or in its bulk. However, their practical application is so far limited by low reproducibility of the electrode properties and limited lifetime of such electrodes caused by the deterioration of the modifying system [96].

6 Conclusions

It follows from the examples given above that electrochemical methods are well suited for monitoring of selected environmental carcinogens. They are simple, cheap, sensitive, reasonably reliable and contain a certain degree of inherent controllable selectivity. Their main drawbacks are connected with their limited scope, problems with passivation of electrode surface and the fact that relatively detailed knowledge about electrochemistry is required for their successful application. Their real strength in this field is their application on easily electrochemically oxidizable or reducible carcinogens. Carbon paste electrodes (eventually chemically or biochemically modified) can be successfully used for the determination of anodically oxidizable chemical carcinogens and mercury electrodes (classical dropping, hanging or chemically or biochemically modified) for the determination of cathodically reducible chemical carcinogens. It seems to us that it is not practical to apply these methods for the determination of electrochemically inactive chemical carcinogens, where they cannot compete with more sophisticated spectrometric (e.g. fluorimetric) or separation (e.g. HPLC with fluorimetric, mass spectrometric or chemiluminiscent detection) techniques.

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