On-line Microdialysis Coupled with Liquid Chromatography for Biomedical Analysis

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Abstract

This work presents a review of the microdialysis (MD) sampling technique for on-line coupling with high-performance liquid chromatography (HPLC) for biomedical analysis. MD-HPLC was first used in the field of brain metabolism to study neurotransmission, and this remains its common application in the biomedical field. On-line MD-HPLC hyphenated methods provide advantages over those with off-line MD-based techniques, including simplified sample preparation, automated analyses, avoidance of contamination introduced during the analytical process, and in situ analysis of the extracellular fluid of living organisms. This review outlines the effectiveness of the continuous monitoring of unbound chemicals from tissues, organs, and body fluids by on-line MD-HPLC methods. In addition, a discussion is presented on the application of in vivo on-line MD-HPLC toward obtaining biochemical event information in the extracellular fluid of various tissues and in biological fluids for pharmacokinetic, pharmacodynamic, toxicological, and bioprocess monitoring.

Introduction

Development of the microdialysis (MD) technique originated from hemodialysis, which acts to eliminate some excess metabolites or toxicants present in the blood (1). Microdialysis is a powerful sampling technique and is used to obtain protein-free samples (2–5). The MD probe, which includes a semipermeable or porous membrane, is the major component of this device. The probe only allows low molecular weight molecules from interstitial fluid to diffuse into the membrane and flow out via the action of a MD pump (6). Fluid coming from this membrane is called dialysate. Owing to its cut off, all medium and high molecular weight molecules (e.g., proteins) are restricted to the membrane exterior.

Recently, microdialysis has become an important technique for continuous in vivo sampling of extracellular fluid in discrete compartments of living systems (7–10). Microdialysis was initially used to monitor neurotransmitter release in the brain. Currently, this technique is also applied in many other fields, including pharmacokinetics, toxicology, and bioprocess monitoring (11). In addition, the microdialysis sample cleanup method has become a powerful technique for studying biochemical events in the extracellular fluid of various tissues and in biological fluids (12).

The development of this analytical technique facilitates the combination of MD sampling, both off-line and on-line, with a suitable detection system. For the separation of materials, this analytical method has been coupled with a separation technique prior to molecular detection. The separation technique, such as high-performance liquid chromatography (HPLC) (13), capillary electrophoresis (CE) (14), and flow-injection schemes (15,16), can be coupled on-line with a suitable detector for the determination of different events. The off-line MD-based technique has been used to overcome some problems resulting from the sample preparation steps required for extraction, or multi-instrument analysis of dialysate (17); however, the operating procedure can sometimes lead to contamination of the analyte.

Abbreviations

3-MT = 3-methoxytyramine
5-HT = 5-hydroxytryptamine
8-OHdG = 8-hydroxydeoxyguanosine
As(III) = arsenite
As(V) = arsenate
ACh = acetylcholine
ASTED = automated sequential trace enrichment of dialysates
DA = dopamine
DHBA = dihydroxybenzoic acid
DMA = dimethylarsinic acid
DOPAC = 3,4-dihydroxyphenylacetic acid
ECD = electrochemical detection
FD = fluorescence detection
HGAAS = hydride generation atomic absorption spectroscopy
HVA = homovanillic acid
MD = microdialysis
MDA = malondialdehyde
MMA = monomethylarsonic acid
PK = pharmacokinetic
TPT = topotecan

On-line MD-based analytical systems can provide many advantages (17–19), such as simplified sample preparation, automated analyses, immediate feedback, minimized sample loss, and avoidance of contamination during the analytical process. In the on-line analytical technique, the MD-HPLC system has been widely applied for continuous in vivo monitoring of unbound drugs and neurotransmitters (17). On-line HPLC with microdialysis perfusion offers some advantages, such as simplified sample preparation and automated analyses. On-line MD-HPLC combined

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with a suitable detector, such as UV–visible spectrometry (UV), fluorescence detection (FD), or electrochemical detection (ECD) (17,19), is used for studying biochemical event information in many fields. These include pharmacokinetics, toxicology, and bioprocess monitoring.

This communication presents an up-to-date report on the available separation pretreatment methods. These schemes have been used for continuous in vivo monitoring of unbound drugs and neurotransmitters. The analysis of endogenous and exogenous substances with various MD-HPLC methods is emphasized. We also provide a pertinent discussion toward the application of a developed MD-HPLC on-line system in the biomedical field.

Applications of On-Line MD-LC Analytical Systems

Extensive studies have shown that MD-HPLC methods are widely used for in vitro experiments and in vivo sampling of analytes for samples of interest, including pharmacology, neurology, biochemistry, and real-time monitoring of biomolecules or other extracellular substances. The following review is organized by the applications of the on-line MD-LC analytical system in biomedical fields, including for the analysis of endogenous and exogenous substances.

Endogenous Substances

In the early 1960s, MD was first employed for sampling free amino acids and other electrolytes in animal brains (20). This technique is a well-established laboratory tool for monitoring brain tissue biochemistry. In recent years, MD has been used in many investigations to measure the concentration of free endogenous substances (e.g., catecholamines, serotonin, amino acids, lactate, pyruvate, peptides, and hormones). Relevant applications of the on-line MD-LC analytical system for analyzing endogenous biomolecules are assembled in Table I.

Table I. Applications of On-line MD-LC Analytical Systems for Determination of Endogenous Substances (The Analytical Method was MD-LC)

Detector	Substance	Species	Model	Reference
FD	Malondialdehy	Brain	Rat	19, 38
FD	Glutamate	Brain	Rat	24
ECD	Dopamine, Serotonin	Brain	Rat	25
ECD	DOPAC, 5-HIAA, HVA, DA, 5-HT	CSF	Rat	26, 27
ECD	DA, 5-HIAA, HVA, DOPAC, L[β-^{11}C]DOPA metabolites	Striatum, Cerebellum	Rat	28
ECD	Melatonin	Jugular vein	Rats	29
FD	Melatonin, metabolites	Pineal gland	Animal	30
ECD	Ascorbic acid	Spinal cord	Rat	32
		Cortex	Rat	35
ECD	8-hydroxydeoxyguanosine	Heart	Rat	39
ECD	Hydroxyl radical	Blood	Rat	40,41,42
UV	Pyruvate, Lactic acid, Ascorbic acid	Striatum	Gerbil	43
ECD	Acetylcholine	Brain	Rat	21
MS	Melatonin	Jugular vein	Rat	31
MS	Enkephalin	Striatum	Rat	36, 37

Acetylcholine

Acetylcholine (ACh) plays an important signal transduction role in both the peripheral and central nervous systems. It is involved in temperature control, blood regulation, motor coordination, learning, and memory, as well as in the controlling stages of sleep. A large amount of research is focused on the role of ACh in neurological diseases. In a recent review by Tsai, various analytical methods for the measurement of ACh have been described (21). For the measurement of Ach, several analytical methods have been developed, including gas chromatography (GC), mass spectrometry (MS), and radioenzymatic assays. However, numerous disadvantages must be overcome, including time-consuming sample collection and clear-up procedures, as well as detection sensitivity and the requisite expensive detectors. Recently, using MD coupled with liquid chromatography–mass spectrometry (LC–MS^2) (22) could obtain most sensitive ACh determination. In a study by Shackman et al., the authors used a CMA/12 probe with a 4-mm length (20 kDa cut off) and the perfusion rate was set at 0.6 µL/min for on-line sampling.

Glutamate

Glutamate plays a key role in the excitotoxicity of secondary brain injuries (23). Glutamate is involved in many neurodegenerative diseases or ischemic tissues. This is due to the damage of glutamate reuptake pathways or the deregulation of glutamate release, thereby causing an increase in glutamate levels. An on-line MD equipped with an HPLC–FD system was used by Yang et al. to monitor glutamate levels in brain ischemic rats (24). In Yang's study, the efficiency of on-line derivation was in agreement with off-line derivation. It is well known that brain ischemia can induce glutamate accumulation. As observed in Yang's study, glutamate concentrations significantly increased after brain ischemia. These results are in good conformity with previous studies. The use of this on-line MD-LC system in Yang's study can also simultaneously perform analyses of other amino acids in the brain, such as asparagine, glutamic acid, glutamine, glycine, Tau, and gamma-aminobutyric acid.

Dopamine, 5-hydroxytryptamine, and their metabolites

Since 1990, MD-HPLC on-line systems have been reported that monitor changes in dopamine (DA), 5-hydroxytryptamine (5-HT), and their metabolites [e.g., 3-methoxytyramine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA)] in brain tissue during ischemia (25). The MD probe was implanted at the forebrain of a rat, and this probe was combined on-line with an HPLC analytical system. Results demonstrated that DA levels increased dramatically during ischemia; however, the concentrations of 3-MT, DOPAC, and HVA decreased by ~ 15–25% of the baseline during ischemia. Chaurasia et al. (26) and Saigusa (27) also used a MD-HPLC-ECD on-line system to monitor DA and 5-HT, as well as their metabolites, in rats. Recently, MD-HPLC, coupled with positron detection, has been developed for animal

metabolism studies (28). In this study, two probes were implanted in the striatum and cerebellum, respectively. This dual-probe sampling technique can reduce the retention time of analytes and produce data in near real-time, thus improving time resolution, minimizing sample manipulation, and simultaneously providing more information about different areas of the same animal.

Melatonin

Melatonin is synthesized from tryptophan via enzyme conversion in the pineal gland and is controlled by a central pacemaker located in the suprachiasmatic nucleus. In early studies, various methods were used to measure melatonin, such as HPLC coupled with ECD or fluorimetric detection. The report of Azekawa et al. (29) in 1990 was the first application of on-line MD sampling coupled with HPLC-ECD toward the study of melatonin in the pineal. In this investigation, the MD probe was constructed in their laboratory (3-mm length, 0.2-mm i.d., 5 kDa cut off). The MD probe was implanted into the guide cannula in freely moving male Wistar rats under a 12 h light-dark cycle. In vitro recovery for melatonin was about 10%, and the melatonin levels significantly increased 2 h after the light was turned off. As observed in their study, on-line MD-HPLC-ECD methods displayed great sensitivity and specificity for melatonin. Recently, Borjigin and Liu (30) developed on-line MD coupled with HPLC-FD to determine melatonin levels in rat brains for circadian rhythm research. The experiment was run continuously for 30 days. Results showed that melatonin levels decreased as the dark period was shortened. An on-line MD-LC–MS–MS analytical system was also applied to monitor melatonin in a freely moving rat for a period of 15 h (31). In this study, the microdialysis probe was surgically implanted in the jugular vein of a male SD rat. Dialysates were measured by LC–MS directly without sample preparation. Results demonstrated that in vivo microdialysis coupled on-line to LC–MS–MS is a promising method for studying pharmacokinetics in a freely moving animal. All of these sensitive and convenient analytical methods would be useful in future applications.

Ascorbate

Ascorbate is an antioxidative vitamin that scavenges free radicals. It is involved in brain damage, cancer, stroke, and arthritis. In order to monitor the dynamic change of ascorbic acid concentrations in anesthetized rats, both before and after an experimental spinal cord injury, an on-line MD-HPLC-ECD analytical system was employed in Tsai's (32) study. In this work, a homemade 170-µm o.d., 150-µm i.d., 3-µm length MD probe was implanted into the rat spinal cord. Microdialysates were injected on-line onto the HPLC column through an on-line injector. All reagents were flowed through tubing to minimize ascorbate contact with air. This procedure avoids ascorbate oxidation and increases the accuracy of determination. The report was a continued study, followed by previous publications (24,33,34), to determine if extracellular glutamate will induce an increase in extracellular ascorbic acid concentrations. Another study by Yang et al. (35) used a similar on-line analytical system for in vivo, continuous, and automatic monitoring of extracellular ascorbic acid in anaesthetized rats. They found that cerebral ischemia significantly raised ascorbic acid levels in the cortex extracellular space, while myocardial ischemia did not significantly change ascorbic acid levels in the left ventricular myocardium extracellular space.

Neuropeptides

MD sampling technique has not been widely used for neuropeptides analysis even through many neurotransmitters are peptides. The extremely low concentration of peptides recovered by MD sampling probes might be a limitation in using MD sampling technique for neuropeptide monitoring. Advances in capillary liquid chromatography coupled with tandem MS are highly attractive for the determination of peptides. These advances have increased the sensitivity, selectivity, and reproducibility of analyses. Baseski et al. (36) studied the variation of enkephalins, which are agonists for opioid receptors, in the striatum of SD rats. Information on the regulation of these is important for pain inhibition and drug addiction research. Enkephalins are produced by the proteolysis of prodynorphin, proopiomelanocortin, and proenkephalin A within neurons. MD provides an index of substance release and degradation in extracellular fluid with a balanced concentration. On the other hand, the tissue measurement is only release, with no change in degradation rates. CMA/12 probes (4 mm in length and with a 20 kDa cut off) were used in this study. In vitro recovery was between 20% and 40%. The infusion of K^+ into the striatum was performed to depolarize neurons and cause an obvious release of enkephalin, both in anesthetized and awake animals, with detection by on-line MD coupled with capillary LC–MS (3). Results showed that an increased flow rate and decreased column rebalance time can accelerate the analytical separation from 30 min to 4 min. A similar study was reported by Shackman et al. (37).

Malondialdehyde

Malondialdehyde (MDA), which is a lipid peroxidation product, is a biomarker of oxidative stress. Yang et al. (19) and Sheu et al. (38) independently developed on-line MD monitoring of MDA, which was coupled with HPLC-FD. Yang et al. applied the analytical system to assess lipid peroxidation in the brain cortex of anesthetized rats. The commercial MD probe (CMA/20) was implanted into the cortex. The microdialysate was derivatised on-line with thiobarbituric acid (TBA), and the mixtures were then automatically injected onto the HPLC column. As MDA is not a stable substance, this on-line analytical system avoids any contact of MDA with air. As observed in Yang's study, MDA production significantly increased after L-trans-pyrrolidine-2,4-dicarboxylate perfusion. Additionally, Sheu et al. used metal-induced lipid peroxidation of fatty acids. In this experiment, a similar on-line analytical system was used for the continuous monitoring of MDA levels.

8-Hydroxydeoxyguanosine

8-Hydroxydeoxyguanosine (8-OHdG) is produced by oxidative damage of DNA via the action of reactive oxygen and nitrogen species, and can be a biomarker for oxidative stress. In a previous study by Yang et al. (39), which used on-line MD-HPLC, it was demonstrated that 8-OHdG levels significantly increased after myocardial ischemia. In this investigation, the MD probe was implanted into the left ventricular myocardium in anesthetized

rats. Subsequently, 8-OHdG levels in the microdialysate were analyzed using an HPLC-ECD system. Additionally, they found that the administration of α-tocopherol acetate significantly suppressed reperfusion-induced 8-OHdG levels. These results may be useful for understanding the protective role of α-tocopherol after myocardial ischemia.

Hydroxyl Radicals

Yang et al. (40, 41) applied on-line MD coupled with an HPLC-ECD to determine 2,3- and 2,5-DHBA in the extracellular fluid of the cortex or striatum. In vitro recoveries were 12% to 20%. Results revealed that elevated concentrations of 2,3- and 2,5-DHBA were correlated with high glutamate concentrations during brain ischemia. Further, MD has also been applied for the measurement of hydroxyl radicals in rat blood vessels (42). According to the authors, the analytical system might help enable further understanding of the mechanism of cell or tissue damage and serve as a diagnostic or even therapeutic monitoring tool.

Other Endogenous Substances

In order to reveal information about endogenous substances, on-line MD-HPLC analytical systems were the most utilized methods. Recently, MD techniques have become one of the major tools for measuring endogenous substances in extracellular spaces. Cheng et al. (43) applied an on-line MD-LC analytical system for the in vivo, automatic, and continuous monitoring of extracellular lactic acid, pyruvate, and ascorbic acid in anesthetized gerbils. As a result, significant and dynamic changes in extracellular pyruvate, lactic acid, and ascorbic acid were detected with isocratic separation within 3 min. According to previous studies, lactic acid levels, pyruvate levels, and the lactic acid-pyruvate ratio in the brain have been advocated for the estimation of stroke severity and also as tools for predicting the outcome. Therefore, the rapid on-line analytical system is a useful method for monitoring extracellular endogenous substances in organs. A similar system has been employed to investigate the dynamic changes of pyruvate and lactate in primary liver cell culture media during hypoxia. As a result, they found pyruvate and lactate levels drastically changed during hypoxia and reperfusion. The application of on-line MD-LC in cell culture media would be helpful for investigations into the roles of these endogenous substances in similar experiments.

The analytical method was successfully used to monitor these endogenous substances after various induced procedures. It can be used to help understand the key roles of various substances.

Exogenous Substances

Another important field of application is the use of on-line MD-LC analytical systems in exogenous substance studies, including the analysis of drugs, chemicals, and heavy metals. This on-line system can be used to monitor the dynamic change of analyte levels and their metabolites. On-line MD-LC would be helpful for understanding the mechanism of these exogenous substances. Relevant applications of the on-line MD-LC analytical system for the determination of exogenous biomolecules are shown in Table II.

Psychotropic Drugs

Psychotropic drugs and their metabolites are the most popular application of MD sampling for therapeutic drugs. In order to understand the dynamic concentration changes of these psychotropic drugs, Tsai (44) and Chen (45) provided an on-line MD-HPLC-ECD system for measuring buspirone and trazone in male SD rats, respectively. Buspirone is an anxiolytic agent and trazone is an anti-depressant drug. Both of these drugs are psychotropic. In these studies, the MD probe was implanted into the striatum of the animal's brain, and Ringer's solution was used as the perfusate. Microdialysate was automatically injected into a microbore HPLC system with an ECD. The low flow rate of the perfusate and mobile phase offered two major advantages, including high sensitivity and low sample volume.

Antibiotic Agents

Antibiotic resistance caused by the widespread use of antimicrobial agents during clinical treatment has already become an increasing worldwide public health problem for bacterial infections. In order to evaluate the possible effect of antibiotic agents, the in vivo monitoring of levels of these drugs is important. Fluoroquinolones are antibacterial compounds and are widely used for human medical applications. Cohen et al. (46) reported the use of on-line MD sampling coupled with on-line microbore HPLC-fluorescence detection for the

Table II. Applications of On-line MD-LC Analytical Systems for Determination of Exogenous Substances (The Analytical Method was MD-LC)

Detector	Substance	Species	Model	Reference
ECD	Buspirone	Striatum	Rat	44
ECD	Trazone	Striatum	Rat	45
FD	Fluoroquinolone, sarafloxacin, oxolinic acid	Liver	Chicken	46
UV	Fluconazole	Blood	Rat	55,56
FD	Topotecan lactone, Carboxylate	CSF	Mice	58
UV	Chloramphenicol, Glucuronide	Blood	Rat	47
UV	Unbound ceftriaxone	Blood	Rat	48
UV	Unbound cefmetazole	Blood	Rat	49
UV	Unbound cephalothin	Blood	Rat	50
UV	Unbound cefepime	Bile	Rat	51
UV	Unbound cephaloridine	Blood	Rat	52
UV	Free cefsulodin	Blood	Rat	53
UV	Meropenem	Bile	Rat	54
FD	20(s)-camptothecin	Bile	Rat	57
UV	Acetaminophen	Blood	Rat	59
UV	Caffeine, paraxanthine,theobromine, theophylline, APAP, APAP-S, APAP-G	Blood	Rat	60
UV	Diclofenac, cyclosporine A	Bile	Rat	62
MS	Contrast agents (Omnipaque)	Carotid vein	Rat	63
HGAAS	Arsenic species (AsIII, AsV, DMA, MMA)	Urine Blood	Rat Rabbit	18 67

measurement of fluoroquinolone antimicrobials in chicken liver. The authors used an automated sequential trace enrichment of dialysates (ASTED) system to clean up and concentrate samples. ASTED has been used for a wide range of water-soluble compounds, including drugs, food additives, toxins, bioamines, vitamins, and pesticides. The dialysis block was constructed like a sandwich, with the dialysis membrane in the middle and the sample extracts in the upper site. This cellulose membrane had a cut off of 15 kDa. Thus, only small molecules could diffuse across the membrane to the lower site. Three antibiotics (i.e., sarafloxacin, oxolinic acid, and flumequine) did not break through the membrane during this process. The analytes concentrated onto the trace enrichment column (TEC) cartridge were injected onto an HPLC column and detected via fluorescence. This MD system avoided sample accumulation on the injector hole. In vitro recovery was 80–113%. This result was due to the use of an MD system, which elevated analytical quality and efficiency.

Other antibiotic drugs have been similarly studied, including chloramphenicol (47), ceftriaxone (48), cefmetazole (49), cephalothin (50), cefepime (51), cephaloridine (52), cefsulodin (53), and meropenem (54). In all of these studies, the on-line MD-HPLC analytical system was performed using rats for the animal model. This analysis system is excellent for providing advanced information about pharmacokinetics and drug metabolism in a small sample volume. For small animals, such as gerbils, it is difficult for investigators to collect enough blood samples throughout the experimental process for a dynamic investigation. Therefore, it is important to develop an appropriate tool to overcome this problem. Furthermore, results from these in vivo studies have provided important information concerning drugs and their metabolites. This information might be extremely useful for the prediction of treatment outcomes in microbial infections, thereby further developing our knowledge about the fate of these drugs.

Fluconazole is an oral antifungal agent that is typically used to treat superficial and systemic candidiasis, as well as to treat cryptococcal infections in patients with acquired immunodeficiency syndrome. There are limited methods to directly estimate drug concentrations in the dermis. Some techniques include the skin blister fluid method, stripping method, and biopsy homogenization. These methods lack reproducibility in pharmacokinetic studies. Further, these techniques only allow the determination of total drug concentration, whereas pharmacological activity relates to the unbound concentration. In the past, some of these methods were applied to pharmacokinetic studies, but they were time-consuming and changed drug activity. Another sensitive method is gas chromatography with electrochemical detection (GC–ECD); however, this technique has laborious sample pretreatment requirements and the validation concentration is too narrow. In 2003, Mathy et al. (55) reported their pharmacokinetic study of fluconazole in dermis and blood by double-site MD coupled with HPLC-UV detection. The authors implanted one CMA-20 probe into the jugular vein, while another probe was implanted into the dermis on the dorsal region of the rat. By using a microbore column (inner radius of only 1 mm), the dialysis time was decreased from 20 min to 12 min, while, in addition, the volumes of solvents and waste were decreased. Moreover, calibration curve slopes of the inter-assay and intra-assay between a microbore column and conventional column were not significant ($p > 0.05$). Other authors have reported a similar study (56), where the difference was a replacement of the vein injection to an application of 0.5% fluconazole gel (0.5 g) to skin in the dorsal region. This determined how much fluconazole was transmitted from the epidermis to dermis. Both of these studies used free moving animals.

Anticancer Agents

Cancer chemotherapy is an important constituent of cancer therapy. Nowadays, there are more than 50 chemotherapy drugs currently available to treat cancer cells. The on-line MD-HPLC technique would be useful for understanding the pharmacokinetic analysis of anticancer drugs. Tsai et al. (57) investigated camptothecin, an anticancer drug and inhibitor of topoisomerase I (57). This highly fluorescent agent can be detected by fluorescence at the nanogram level. A 7-cm dialysis membrane was implanted into the common duct, a normal elimination site of drugs. This on-line analysis system was composed of MD sampling, microbore HPLC separation, and detection via FD. In vivo recovery was achieved by retrodialysis and the result was 87.12%. Compared to other studies for bile fluid collection, MD sampling offers a simple sampling process, avoids bile loss, avoids matrix interference, and decreases the number of sacrificed animals. Topotecan (TPT) is a camptothecin analog and a second line therapy in metastatic ovarian cancer that interacts with topoispmerase I (58). A probe (1-mm length) having a dialysis membrane was implanted into the lateral ventricle of a TPT-treated mouse. Owing to dimensional considerations, it is difficult to implant the probe at the correct location to avoid sampling errors. With this small probe, inaccuracy in achieving the correct implantation site was reduced and caused minimal physical damage to the tissue. The perfusate rate was lowered to 0.5 µL/min to obtain adequate probe recoveries of 5% to 15%. Results of this study were obtained continuously with various time intervals of the active TPT form detected by the MD sampling system. This is in contrast to the total amount from brain homogenate that is analyzed by conventional extraction.

Chemicals

Arbutin, ascorbyl glucoside, kojic acid, hydroquinone, and niacinamide are commonly used bleaching agents in bleaching cosmetics (64–66). Arbutin prevents serious sunburn caused by the accumulation of melanin in subcutaneous tissue. For bleaching cosmetics, the maximum authorized concentration of arbutin is 7%. Isolation of bleaching agents from the matrices mentioned here, prior to quantitative analysis, requires several stages, including sonication with acids, centrifugation, filtration, and solvent extraction. Because these processes are laborious, time-consuming, and involve large volumes of hazardous solvents, they are not suitable for the routine analysis of commercial products. The authors developed a simple, reliable, and fast on-line MD–HPLC method. This method uses a low amount of organic solvent to quantitatively determine bleaching agents in bleaching cosmetics. Recoveries were in the range of 92% to 106%, with good reproducibility.

Heavy Metals

Arsenic is a well known toxicant. The inorganic arsenic, when present as arsenite [As(III)] and arsenate [As(V)], is highly toxic. Some methylated arsenic species, such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), are only moderately toxic, and they are considered part of some detoxification mechanisms in living organisms. Urinary arsenic species are not stable when exposed to ambient air. In order to obtain a real time, in situ analysis of urinary arsenic species in the bladders of living organisms, on-line MD coupled with HPLC would be a suitable method. A fully automated method for continuously monitoring As(III), As(V), MMA, and DMA using an on-line MD-HPLC-Hydride generation atomic absorption spectrometry (HGAAS) system was developed by Tseng et al. (18). The MD probe was first implanted into the bladder of a rat. In vitro recovery was excellent, being measured at 98% to 105%. Moreover, a similar on-line system was developed for the continuous monitoring of As species in the whole blood of rabbits (67). With intravenous injection of arsenite trioxide, the limit of detection for the As(III), As(V), MMA, and DMA were 2.0, 2.9, 2.4, and 4.2 ng/mL, respectively.

Others

The development of an on-line MD-microbore LC method for the determination of acetaminophen, caffeine, theobromine, paraxanthine, tirapazamine, and its reduced metabolites in rats has been described (59–61). In this analytical system, developed for the study of pharmacokinetics, the perfusion rate, sample volume, retention time, and required temporal resolution of the experiment were all interrelated. Results from these experiments demonstrated that the present method offers great limits of detection and quantitation, in addition to good precision and reproducible accuracy. The authors demonstrated that the on-line analytical system has been successfully applied in an awake and freely moving animal. Furthermore, a similar analytical system has also been applied for the in vivo analysis of diclofenac and cyclosporine A (62). Diclofenac is a nonsteroidal anti-inflammatory agent, which decreases the formation of prostaglandins. The authors compared their analysis system with others, such as HPLC–UV, HPLC–ECD, GC–MS, HPLC–FD, GC–MS, and HPLC–ICP-MS. The above LC-based methods determined the total amount of diclofenac, and were not able to assess unbound drugs. The analytical on-line MD system was successfully applied for the determination of diclofenac in rat bile, and the in vivo microdialysis recoveries were between 69 and 73%. Moreover, the on-line MD-LC analytical system was also applied for the in vivo analysis of a contrast agent (63). In the last decade, mass spectrometers (MS) coupled on-line to HPLC have provided powerful tools for the rapid analysis of a wide variety of organic compounds in complex biological matrices.

Summary

We have shown that microdialysis sampling combined on-line with HPLC analytical methods can be used effectively to monitor endogenous and exogenous substances in the biomedical field. On-line MD-HPLC techniques provide more information for pharmacological, neurological, biochemical, and real-time monitoring of biomolecules by in vivo sampling of extracellular fluid, for many kinds of tissues and fluid. The advantages of these on-line systems are as follows: sampling rates close to real time, improved temporal resolution of the sampling time, reduced sample pretreatment before HPLC separation, and simultaneously obtained information for an animal. Furthermore, MD-HPLC serves as a research tool that aids the in vivo analysis of endogenous and exogenous substances in the fields of pharmacokinetics, neurology, toxicology, and bioprocess monitoring.

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