Review Article

Drug abuse analysis using high-performance liquid chromatography with monolithic columns – A review

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ABSTRACT

The problem of drug abuse has become one of the toughest problems for governments around the world. Based on the cases of drug abuse, a method of drug abuse analysis is needed to monitor its levels in the body. Several methods have been developed, but the most widely used is HPLC. The application of HPLC method requires fast and inexpensive testing techniques. In the past, particle columns were used for analysis many compounds. However, because of the tendency for blockages and backpressure to be very high, monolithic columns are used as an alternative for those problems. There are many applications and development of monolithic columns for drug abuse analysis. Monolithic columns are used for separation substance of drug abuse in their matrices. Based on the research that has been done, monolithic column shows better results compared to particle columns such as high selectivity, low cost, easy and simple preparation, and also minimizing high backpressure.

KEY WORDS: Drug abuse analysis, Hybrid monolithic column, Monolithic column, Monolithic polymer column, Monolithic silica column

INTRODUCTION

The problem of drug of abuse (DOA) has become one of the toughest problems for every government around the world. In 2011, there were around 49% of DOA cases out from a total of 5.1 million during visits to the emergency department in United States. In 2010, DOA had caused 99,000–253,000 deaths globally[1] and increased to about 60% from 2000 to 2015.[2] Cocaine, heroin, and similar drugs cause around 0.2 million deaths each year.[3] DOA is aimed at compounds that have psychoactive properties, where illegal use can cause addiction. However, so far there is no apparent difference between DOA and drugs that have a therapeutic effect. On the one hand, many compounds are generally categorized as DOA substances but have therapeutic effects. Groups of drugs considered DOA are compounds that are used without prescription or used with excessive doses. Drugs such as benzodiazepines, ketamine, and opioids have a significant potential for abuse even when used in the treatment of certain diseases. The effects of DOA are not only for the individual themselves but also their family and environment.[4] In Western, the number of perceptions of the low risk of danger makes cannabis can be easily found. The use of some drugs is often preceded by the previous use of marijuana. Abuse of illegal drugs many found in teenagers. In high-income countries, ecstasy, methamphetamine, cocaine and ketamine are often usually used while inhalational drugs used by young street people.[2]

Based on the number of cases of DOA, methods for the analysis of drugs that often and have potential to be misused are needed to monitoring the levels in the body. Several methods have been developed in terms of analyzing these drugs, for example, using high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC-MS), and liquid chromatography–mass spectrometry (LC-MS).

Initially, the particle column was widely used for compounds analysis. For example, in 2010, Berset *et al*. used silica particle columns for analysis of illicit drugs in water samples.[5] Bjørk *et al*. also analysis drug of abuse and metabolites in whole blood.[1] Another research has been done by Baker and Kasprzyk-Hordern for analysis of DOA in wastewater.[6] In 2011, Baker and Kasprzyk-Hordern also used particle columns for analysis of drug of abuse from waste and

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surface water samples.[6] In 2012, particle column also used for the analysis of DOA in human urine.[7]

Because of its use, which can increase the volume of mobile phase and frequent emergence of high backpressure, the monolithic column is used as another alternative. Monolithic columns can produce efficient separation with high flow rates and low backpressure. In addition, accuracy, sensitivity, and speed of analysis can also be achieved.[8,9]

DISCUSSION

Analysis of DOA Substances

The use of illegal drugs is rise in the community. Therefore, analysis is needed to see the possibility of exposure to these drugs in the body. This analysis is usually carried out through two stages, namely screening and confirmation tests. At the screening stage, many immunoassay enzymes are used. After the positive compound was detected, the analysis continued using GC-MS. GC-MS was chosen because of its selectivity and sensitivity. However, considering the high costs required and the need for a sample derivation process first, application of GC-MS is limited. The use of GC-MS is displaced by the presence of HPLC, where the HPLC method can be used in a broad spectrum compound, not depending on the polarity and volatility of the compounds to be analyzed. The use of HPLC requires fast and inexpensive testing techniques. In the past, particle columns were used for analysis. However, because of the tendency for blockages backpressure to be very high, a monolithic column is used as an alternative.[10]

The monolithic column is taken from Greek, namely "Monos" and "lithos," where the full meaning is a column consisting of massive stone. These columns are made with various pore sizes, ranging from micropore (<2 nm), mesopore (2–5 nm), and macropore (>50 nm).[11] Many monolithic columns are developed as a substitute for conventional particle columns because their superiority.[12] The main difference between the monolithic column and particle column lies in the necessary arrangement of the structure. Conventional particle columns consist of several porous particles with micro sizes, which are located close together. This causes fluid is challenging to be penetrated. Although, in this case there is a process of diffusion or transport of molecules to be separated into active sites in the pore and then back to the mobile phase, the process takes a long time especially for large molecules. Success of separation in that process depends on how long sample can stay in the stationary phase. While, monolithic column consists of only one part of porous material, and it can be used at low flow rate. The separation efficiency can be increased by increasing the flow rate, gradually.[13]

Based on the constituent components, this monolithic column is divided into three types, namely natural or inorganic monolithic (the constituent is silica), organic monolithic (the constituent is polymer), and hybrid monolithic (a mixture of inorganic and organic materials).[14]

Silica-based Monolithic Column

The silica-based monolithic column is an inorganic monolithic made from single porous silica with various processes.[15] In general, this silica monolithic has a good separation profile although in a short analysis time compared to conventional particle columns.[8]

Hybrid Base Monolithic Column

The hybrid base monolithic column is a combination of the use of organic and inorganic materials. It consists of two types, namely silica-hybrid base monolithic column and polymer-hybrid base monolithic column. This type of monolithic column has potential for development, considering its composition in a flexible material that has biocompatibility and excellent mechanical properties can also be used and stored in a long time, relatively.[16]

Polymer-based Monolithic Column

The selectivity problem in non-imprinting monolithic columns (non-imprinted polymer [NIP]) often causes the results of analysis to be unsatisfactory. NIP produces a much lower recovery compared to the molecularly imprinted polymer monolithic column (molecularly imprinted polymer [MIP]).[17] For analysis substances with small amounts, MIP monolithic column is vital. This is due to the binding ability of the template and target molecule from MIP, which NIP does not have.

The polymer base monolithic column is a technique of making polymer-based on bonds that occur between molecular templates with individual binding sites.[18] With this bond, the polymer produces have an excellent ability to recognize target molecules, both physically and chemically.[19] Polymer base monolithic columns can be used as separation techniques for biological samples with complex matrices.

Monolithic column based on molecularly imprinting as a stationary phase is carried out by *in situ* method, where the polymer mixture is put into a stainless steel column.[20] This composition consists of template, as prints of the analysis compounds. Porogen as a solvent that can form pores, functional monomer, cross-linker, and initiator.[21] This easy and fast preparation can be directly connected to HPLC instrument.[22] Preparation using this technique can also save costs because it uses a small number of templates.[17]

Compared to conventional polymer, this monolithic polymer column has many benefits because of its simple preparation which does not involve sieving and grinding process that can be time consuming and allows the same size and shape particle.[23] In addition, the polymer base monolithic columns have high permeability due to the presence of porous structures formed by porogens which allow manipulation of flow rates when applied to HPLC instrument, and separation efficiency can be reached.[24] Another advantage of this type of monolithic column such as easy preparation process tends to be resistant to various conditions and low cost. The success of analysis can also be achieved by the existence of specific binding site between template and target molecule.[22]

Monolithic Column Application for Analysis of DOA

The many cases in DOA encourage researchers to create a good analytical method to monitor its level in the body. The monolithic column has been widely applied, both for analysis in pharmaceutical analysis, food contaminants, and amino acid or protein.[8,14,25] The application of the monolithic column for an analysis of drugs that are often misused is shown in Table 1.

Application of Silica-based Monolithic Column

The use of silica-based monolithic columns has been used to analyze benzodiazepines drugs in whole blood samples, including the eight compounds found in forensic toxicology. This analysis found at least 30 compounds from two real cases, with an analysis duration is <4 min. The validation method shows satisfactory results.[26]

Analysis of pseudoephedrine and dextromethorphan in pharmaceutical formulations has also carried out. Hadad *et al*., in their study, compared the conventional silica-based particle column with a silica-based monolithic column for analysis compound. The results show that the monolithic column has higher accuracy and precision, with much faster analysis time than the conventional particle column. In other words, the monolithic column is sensitive enough to detect the drug content in pharmaceutical preparations.[36]

Simultaneous analysis of butalbital in human serum has been carried out. The study by Pistos *et al*. using monolithic silica column combined with solid-phase extraction (SPE) resulted that linearity is excellent and percentage of recoveries reaching approximately 99%.[13]

Analysis using a silica-based monolithic column has also been applied to propranolol. Deeb *et al*. compared the monolithic silica column with conventional particle column. The efficiency and rapid time analysis are indicated by monolithic column where this method produces excellent linearity and recovery for analysis propranolol in pharmaceuticals.[9]

Table 1: Monolithic column for drugs abuse analysis

Drugs	Type of monolithic column	Sample	Year
Heroin	Silica		2004[27]
Butalbital	Silica	Human serum	2004[13]
Benzodiazepines	Silica	Human blood	2004[26]
Ketamine's and its metabolites	Silica	Human plasma	2005[28]
Ephedrine	Polymer		2006[23]
Propranolol	Silica		2006[9]
Ecstasy	Silica	Tablets	2006[29]
Propranolol	Silica	Pharmaceuticals	2007[30]
(S)-Naproxen	Polymer		2007[20]
16 of illicit drugs	Silica	Money	2007[31]
Amphetamine	Silica	Human urine	2009[32]
Methamphetamine			
3,4-methylenedioxyamphetamine			
3,4-methylenedioxymethamphetamine			
10 of antidepressants	Hybrid	Human urine and plasma	2010[33]
10 of antiepileptic	Silica	Human serum and plasma	2010[34]
Salbutamol	Polymer		2010[35]
Terbutaline			
Benzocaine			
Cocaine			
Heroin			
Pseudoephedrine	Silica	Pharmaceutical formulations	2011[36]
Amphetamines	Silica	Human urine	2013[37]
Opiates			
Cannabinol			
Antidepressants	Hybrid	Human plasma	2015[38]
Anticonvulsants			
Anxiolytics			
Psychotic			

The application of silica-based monolithic column is also used for amphetamine and its derivates analysis using GC-MS in urine sample. The analysis was carried out on four compounds that are susceptible to abuse. The results show that the use of silica-based monolithic spin column is suitable for analysis using GC-MS. This method is considered natural and does not require a large volume of solvents for the extraction process. In addition, it can be used simultaneously for the derivation and extraction process.[32]

Another application of silica-based monolithic column is used for the analysis of antiepileptic drug classes. Heideloff *et al*.'s study of 10 antiepileptic drugs in human plasma and serum samples using HPLC show results in improved sensitivity and resolution compared to conventional particle columns. And also, the time for analysis process is shorter.[34]

An analysis of 16 illicit drugs has been carried out. The study was conducted by comparing the efficiency of the silica base monolithic column with conventional particle column. Obviously, application of LC-MS/MS monolithic column provides high sensitivity with relatively fast analysis time. Simple preparation of samples has been developed and applied to illicit drug contaminants in circulating drugs in Ireland.[31]

In addition, the application of silica-based monolithic column has also been used in the analysis of heroin from black market. The chromatogram results show a good separation of heroin. Silica-based monolithic column proved to be a tool that can be used to prepare a fast and straightforward method with good result for heroin analysis.[27]

The silica-based monolithic spin column has also been used for extracting amphetamines, opiates, and cannabinol from urine sample. This column is applied with GC-MS instrument. The results show advantages of this method compared with conventional liquid-liquid extraction and SPE techniques. It is simply and easy technique, and also can eliminate other contaminations allowing sensitive analysis with limit of quantification and limit of detection values at the nanogram levels.[37]

Application of Hybrid-based Monolithic Column

Application of hybrid-based monolithic column for drug analysis of anxiolytics, antidepressants, anticonvulsants, and antipsychotic has also done. Domingues *et al*. carried out on 16 drugs on the plasma sample from schizophrenic patients using an organic-inorganic hybrid cyanopropyl based monolithic column. This hybrid-based monolithic column is selective and shows good absorption ability for that drugs analysis. High permeability and low backpressure from the monolithic column help during the percolation process. In addition, this hybrid-based monolithic column can be applied more than 100 times and it also can be used for therapy drug monitoring for schizophrenic patients.[38]

Other application of hybrid monolithic column is also applied for the analysis of 10 antidepressants drugs in elderly patients plasma and urine sample. The results of linearity are good, which is above 0.99 with recovery reaching 113%. Analysis that utilizes this hybrid-based monolithic column has been successful for antidepressants drug.[33]

Application of Polymer-based Monolithic Column

Polymer-based monolithic column has been widely used as a substitute for silica-based monolithic column. This is due to its high permeability and resistance to various pH condition.[24]

The application of polymer-based monolithic columns has been developed for the separation and evaluation of ephedrine using methacrylic acid (MAA) as a functional monomer, ephedrine as template, and ethylene glycol dimethacrylate (EGDMA) as cross-linker. In a study conducted by Yingchun *et al*., they separated the – (–) ephedrine and + (+) ephedrine molecule by comparing the effectiveness of polymeric and non-polymeric base monolithic columns. The results show that the monolithic polymer column selectively separates – (–) ephedrine and + (+) ephedrine. Therefore, this polymer-based monolithic column can be an alternative to the separation study.[23]

Polymer-based monolithic column has also been applied to naproxen. Research by Ying *et al*. used (S)-naproxen as a template with combination of functional monomers, namely butyl methacrylate and MAA. The combination of this functional monomer results in higher efficiency than the single use.[20]

The advantages of using other polymer-based monolithic column are shown in Cheng *et al*. study for analysis β2-blockers and narcotics drugs. This method has been used for the analysis of several doping agents and has been applied directly to urine samples. Pressurized capillary electrochromatography-ele ctrospray ionization-mass spectrometry method base on monolithic column polymer can be used to control doping agents with a fast and simple method. Compared to traditional methods, this method has several advantages including applied of small volume of sample and mobile phase that allows reduction of contamination to environment, simply pre-treatment sample and avoiding time-consuming derivation.[35]

In addition an analysis of tramadol has also been successfully carried out. Javanbakht *et al*. study applied online solid-phase extraction using polymer base monolithic column for the analysis of tramadol

in human plasma and urine sample. The monolithic column is made by *in situ* technique, where MAA is used as a functional monomer, EGDMA as cross-linker and chloroform as porogenic solvent. The results show good recovery which is reach 90% above. The monolithic column also showed good selectivity for tramadol analysis when adding dextromethorphan, o-desmethyltramadol, and timolol to the sample. Thus, the polymer-based monolithic column can be applied to samples that have complex matrices such as human plasma and urine.[17]

CONCLUSION

All types of monolithic column can be applied for analysis of DOA, for example, narcotics and psychotropic groups. When compared with conventional particle columns, this monolithic column has many advantages such as shorter time analysis, easy and simple of sample preparation, and minimizing high backpressure. The use of monolithic column can also be applied to complex matrices such as human serum, plasma, and urine sample with high sensitivity and accuracy.

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