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What Type of Research Is Done in the Laboratory of Pharmaceutical Analytical Chemistry of the University of Geneva?

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Abstract. This article presents briefly the main research projects developed in the Laboratory of Pharmaceutical Analytical Chemistry at the University of Geneva. This laboratory is particularly concerned about enantiomeric separation of drugs and phytochemical analysis. In this context, techniques such as gas, liquid and supercritical fluid chromatography as well as capillary electrophoresis are studied. Furthermore, the development of new sample preparation techniques is investigated.

1. Introduction

The laboratory of pharmaceutical analytical chemistry (LPAC) was created in 1992 and is part of the pharmacy section at the University of Geneva. In 1995, the phytochemical analysis research group joined the LPAC.

Two main research areas are currently being developed: phytochemical analysis and drugs analysis. Increased coherence in research programs between the two groups is developing, in particular through joint Ph.D. or postdoctoral projects. Overall interdisciplinarity in drug research is continuously emphasized as a priority issue in the further development of all research programs. Eighteen people are involved in the LPAC, among them eight Ph.D. students, two post-doc, one chemist, four technicians, one secretary, one lecturer (MER), and one professor. The laboratory is responsible for the education of 2nd cycle pharmacy students in phytochemistry and pharmaceutical analysis.

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The LPAC has developed many contacts with private, public, and academic laboratories in Switzerland, in Europe, and in South America. It is also involved in European educational projects such as the *Erasmus-Socrates* exchange program.

Since 1996, the laboratory is member of the center of competence in chemical and toxicological analyses (CCCTA, see article in this issue).

For more information about the LPAC, please type http://www.unige.ch/sciences/pharm/fanal/.

2. Research Activities on Chirality

The LPAC has orientated its activities toward the development of separation methods for the analysis of drugs, drugs of abuse and metabolites in several matrices, such as pharmaceutical formulations and biological fluids. In this context, chiral separation methods are particularly studied.

Recently, a regulatory policy was edited by several countries to manage the development of new chiral compounds. Since then, two enantiomers are considered as two different substances and all tests necessary to register a new compound have to be done on both forms and even on the racemic mixture. This growing interest in drug stereochemistry has initiated a tremendous development of analytical methods for the determination of enantiomeric proportions.

Several analytical procedures have been developed for the analysis of enantiomers. Among them, chromatographic and electrophoretic methods are now the most widely used because of their speed, precision, tolerance of impurities and other products in the sample. In our laboratory, we are developing capillary electrophoresis methods and liquid chromatography on chiral stationary phases.

2.1. *Capillary Electrophoresis*

Capillary electrophoresis has greatly developed in the last few years. This is because it possesses a high separation efficiency in comparison to chromatography and it presents numerous advantages such as its rapid method development, its low consumption of polluting solvents, its high speed of analyses and its small need injection volume. Furthermore, the coupling with mass spectrometry has been recently commercialized allowing to obtain a great selectivity and sensitivity.

Separation of amphetamines and more precisely methylenedioxy-derivatives was developed in our laboratory by capillary electrophoresis using β-cyclodextrins as chiral selectors [1][2]. Amphetamines have a potent central nervous system stimulating effect. Among them, amphetamine and methamphetamine have been accepted for their medical usage for years, whereas methylenedioxymethamphetamine (MDMA or Ecstasy) and other methylenedioxy-derivatives such as MDA and MDEA are considered as drugs of abuse and are now commonly used. All of these compounds possess at least one asymmetric carbon and pharmacological studies have shown that (*R*)- and (*S*)-enantiomers have different activities. Enantioresolution of the five most commonly consumed amphetamines can be achieved by this method (*Fig. 1*). The capillary electrophoresis procedure allows us, in this particular case, to drastically reduce our analytical cost. The method is successfully applied in our laboratory on real toxicological cases. This project is conducted in collaboration with the forensic institute of Lausanne.

Studies are now in progress in order to evaluate several kinds of chiral selectors as well as to compare capillary zone electrophoresis with micellar electrochromatography and capillary electrochromatography.

2.2. *Liquid Chromatography*

Liquid chromatography using a chiral stationary phase is now considered as the method of choice for the analysis of trace level compounds in biological matrices.

Approximately 80 chiral stationary phases are now commercially available and can be classified according to the nature of the selector and the involved interactions.

We are studying the behavior of various chiral stationary phases. In this context, the enantioseparation of methadone [3] was developed. Methadone is widely used as a substitute in heroin addicts maintenance programs because it possesses a long half-life and can be administered orally, limiting viral infection problems. Methadone possesses one asymmetric carbon. It is well known that (*R*)-methadone is 50 times more potent than (*S*)-methadone. However, in most countries, methadone is administered as the racemate.

The enantioseparation of methadone was validated after having tested various chiral stationary phases. The selected method (*Fig. 2*) is now applied to sera from patients who are receiving daily racemic methadone. Various projects, such as the evaluation of the pharmaceutical form in the treatment of heroin addicts, are conducted in collaboration with the Phenix Foundation in Geneva and the Drugs of Abuse Division of the Geneva Hospital.

3. Research Activities on Plant Products

Plants produce a broad range of secondary metabolites, a number of which are being used in modern medicine as drug of prime importance. The phytochemical analysis research group is focusing on two major research areas: the first one is the development of *in vitro* tissue culture, in particular of genetically transformed root culture; the second is the development of analytical methods for the determination and the detection of natural compounds.

3.1. In vitro *Tissue Culture*

The understanding of the regulatory processes in the biosynthesis of pharmaceutically important tropane alkaloids and related compounds is being investigated in order to improve their production in plant tissue cultures. The large-scale culture of plant tissue and the modeling of growth of tissue cultures are being studied. Experiments on the production of tropane alkaloids with *Agrobacterium*-mediated transformed root cultures of *Datura candida* x *D. aurea* show that the biosynthesis of the alkaloids may be superior to that of the parent plants. This is achieved through the optimization of the culture medium [4], the addition of nitrate, upon elicitation with chitosan, by the addition of thiamine and by feeding the cultures with (*R,S*)-phenyllactic acid. Furthermore, studies with permeabilizing agents in bioreactors demonstrated the possibility to selectively release one compound of interest into the growth medium.

3.2. *Development of Analytical Methods*

The implementation of capillary electrophoresis in the analysis of tropane alkaloids was developed. Successful results were obtained after optimization of the electrophoretic parameters for the determination of atropine, homatropine, and scopolamine in ophthalmic solutions [5]. Furthermore, micellar electrokinetic capillary chromatography (MEKC) proved to be an excellent tool for the analysis of tropane alkaloids in complex plant extracts [6].

In continuation of our investigations on different *Erythroxylum* species from South America, gas chromatography coupled to mass spectrometry proved to be a powerful technique for the analysis of tropane alkaloids and derivatives [7]. Further identification of new compounds extracted from chemically uninvestigated species is in progress and could help in the understanding of the biosynthetic pathway of this pharmacologically important class of metabolites.

The chemistry of calystegines, a new class of *nor*tropane derivatives which occur in the plant families *Solanaceae* and *Convolvulaceae* is under investigation. These compounds have significant activity towards glycosidases and could present a potential benefit as anti-viral agents. Our group is currently developing simplified analytical procedures to rapidly detect calystegines in the different plant extracts of interest.

Finally, the determination of artemisinin, an antimalaric compound isolated from *Artemisia annua* and artemisinic acid, its major precursor, was achieved. Supercritical fluid extraction (SFE) was developed and optimized and the extracts were analyzed by supercritical fluid chromatography (SFC). The detection of the compounds was carried out by an evaporative light scattering detector (ELSD) [8]. SFE-SFC-ELSD showed that this method allowed mild extraction conditions and quantitative determination without further purification of the plant extract [9].

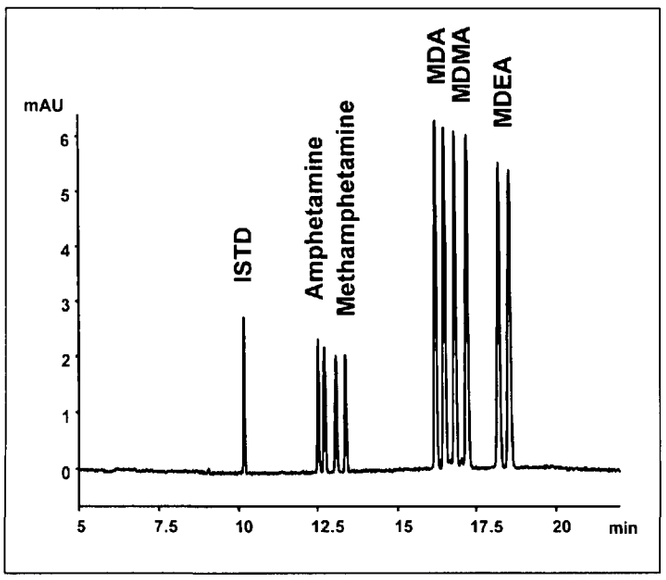

Fig. 1. *Separation of five amphetamines by CZE-UV using the (2-hydroxy)propyl-β-cyclodextrin as chiral selector* [1]

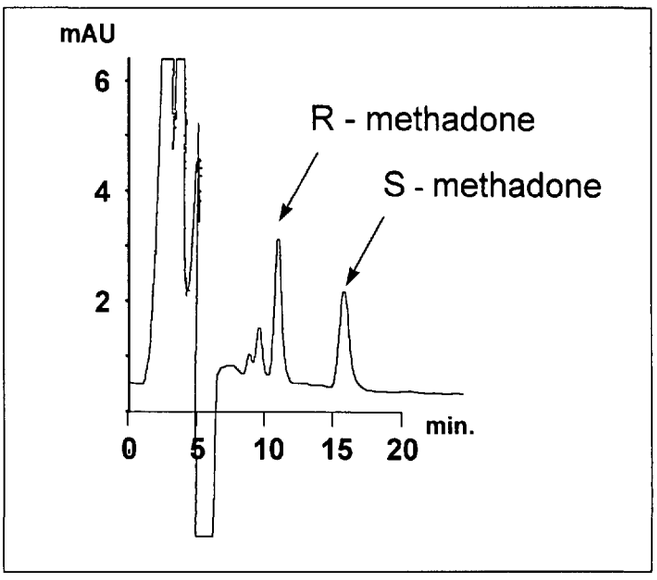

Fig. 2. *HPLC-UV analysis of human serum extracts using a Chiral-AGP column* [3]

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4. Future Developments

Our laboratory is continuing to study new chiral selectors for capillary electrophoresis and stationary phases for liquid chromatography. These new tools will have to be more selective and more rugged to analyze in routine complicated samples such as biological matrices, especially in the case of liquid chromatography.

Furthermore, the application of capillary electrophoresis and electrochromatography as well as μ-liquid chromatography in chiral analysis and in phytochemistry is under development in the LCAP. These powerful methods will permit to miniaturize the total analytical system and reduce the consumption of organic polluting solvents.

The coupling of separation methods with all kinds of selector and the development of new sample preparation techniques is needed to enhance selectivity and sensitivity. Finally, our laboratory is involved in the use of the mass spectrometry coupled to capillary electrophoresis, gas and liquid chromatography.

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[1] E. Varesio, J.-L. Veuthey, *J. Chromatogr., A* **1995**, *717*, 219.
[2] E. Varesio, J.Y. Gauvrit, R. Longeray, P. Lantéri, J.-L. Veuthey, *Electrophoresis* **1997**, *18*, 931.
[3] S. Rudaz, J.-L. Veuthey, *J. Pharm. Biomed. Anal.* **1996**, *14*, 1271.
[4] P. Nussbaumer, I. Kapetanidis, P. Christen, *Plant Cell Rep.*, in press.
[5] S. Cherkaoui, L. Mateus, P. Christen, J.-L. Veuthey, *J. Chromatogr., B*, **1997**, *696*, 283.
[6] S. Cherkaoui, L. Mateus, P. Christen, J.-L. Veuthey, *Chromatographia*, in press.
[7] A. Brachet, O. Muñoz, M. Gupta, J.-L. Veuthey, *Phytochemistry*, in press.
[8] M. Kohler, W. Haerdi, P. Christen, J.-L. Veuthey, *J. High Resol. Chromatogr.* **1997**, *20*, 62.
[9] M. Kohler, W. Haerdi, P. Christen, J.-L. Veuthey, *Phytochem. Anal.*, in press.

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Chemical Analysis in the Life Cycle of Products

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Abstract. Chemical analysis has an important function in the life-cycle of consumer goods and technical products. In this paper the variety of analytical chemistry provided at EMPA St. Gallen is shown by three typical examples concerning the two product categories plastic packaging materials and textile detergents. In addition EMPA is engaged in an overall promotion of the accuracy and reliability of amount-of-substance data.

1. Introduction

Large numbers of consumer goods and technical products are a constant part of our daily lives. We expect these products to be there for our benefit and that they in no way cause us any harm or injury either when they are being produced, during use or during the disposal phase. Irrespective of whether they are made of naturally produced biological material or synthetic material, all such products consist of a combination of basic chemical elements whose characteristics are determined by their chemical composition.

Chemical analysis has a central function in the total life cycle of a product (*Fig. 1*). It is an important instrument when carrying out market research and it also provides support for technical research and development of a product. Chemical analysis is used to characterise raw materials for manufacture and to control production processes. It helps to determine the optimum application range of a product and provides information on possible harmful side effects to man and the environment during the useful phase (*e.g.* release of harmful substances). It also provides information for deciding whether or how a product can be disposed of, whether it can be recycled, incinerated, composted or whether it has to be deposited at a landfill site.

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2. Chemical Analysis at EMPA St. Gallen

EMPA St. Gallen is engaged in the chemical analysis of consumer goods and various technical products. Special attention is paid to packaging material, plastic products, washing and cleaning agents, wood preservatives, paints, varnishes and several more. The diversity of chemical analyses necessary for characterising such products can be illustrated by three examples.

2.1. *Plastic Packaging Material (Examples 1 and 2)*

The analysis of a plastic packaging includes the characterisation of the plastic itself using polymer or structure analysis methods such as SEC, TMA, DSC, FTIR, and also determining qualitatively and quantitatively the presence of organic and inorganic additives such as antioxygenes, UV stabilisers, softeners, colorants, filling material, and also trace elements. Usually chromatographic separation methods are used such as HPLC and GC coupled with suitable sampling and detection technologies (pyrolysis, head space or DAD, FID, MS).

Example 1: The Analyst as Detective

Very often investigations have to be carried out due to cases of damage. For instance, in a recent case consumers complained to a brewery that its beer had a strange smell and that it was not drinkable. Very soon the plastic bottle top came under suspicion. It was believed that trace compounds could have contaminated the beer. The GC/MS analysis indicated among others the presence of tribromphenol and tribromanisol. But where did these compounds come from? After systematic