

CHROMATOGRAPHIC TECHNIQUES IN PHARMACEUTICAL ANALYSIS IN POLAND: HISTORY AND THE PRESENCE ON THE BASIS OF PAPERS PUBLISHED IN SELECTED POLISH PHARMACEUTICAL JOURNALS IN XX CENTURY

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Abstract: For a long time, chromatographic techniques and techniques related to them have stimulated the development of new procedures in the field of pharmaceutical analysis. The newly developed methods, characterized by improved metrological parameters, allow for more accurate testing of, among others, the composition of raw materials, intermediates and final products. The chromatographic techniques also enable studies on waste generated in research laboratories and factories producing pharmaceuticals and parapharmaceuticals. Based on the review of reports published in Polish pharmaceutical journals, we assessed the impact of chromatographic techniques on the development of pharmaceutical analysis. The first chromatographic technique used in pharmaceutical analysis was a so-called *capillary analysis*. It was applied in the 1930s to control the identity of pharmaceutical formulations. In the 1940s and 1950s, the chromatographic techniques were mostly a subject of review publications, while their use in experimental work was rare. Paper chromatography and thin layer chromatography were introduced in the 1960s and 1970s, respectively. These new analytical tools have contributed to the intensive development of research in the field of phytochemistry and the analysis of herbal medicines. The development of column chromatography-based techniques, i.e., gas chromatography and high performance liquid chromatography took place in the end of 20th century. Both aforementioned techniques were widely applied in pharmaceutical analysis, for example, to assess the stability of drugs, test for impurities and degradation products as well as in pharmacokinetics studies. The first decade of 21st century was the time of new detection methods in gas and liquid chromatography. The information sources used to write this article were Polish pharmaceutical journals, both professional and scientific, originating from the interwar and post-war period, i.e., „Kronika Farmaceutyczna”, „Farmacja Współczesna”, „Wiadomości Farmaceutyczne”, „Acta Poloniae Pharmaceutica”, „Farmacja Polska”, „Dissertationes Pharmaceuticae”, „Annales UMCS sectio DDD Phamacia”. The number of published works using various chromatography techniques was assessed based on the content description of individual issues of the journal „Acta Poloniae Pharmaceutica”.

Keywords: chromatography, history, pharmaceutical analysis

It is not without a reason that the 20th century was named a *century of separation science* (1). After more than a hundred years of the development of chromatographic techniques, analytical chemistry has gained a tool without which knowledge about the environment, food and natural products would have been much poorer. The opportunities created by chromatographic techniques are also used by professionals involved in pharmaceutical analysis, who often study samples characterized by complex composition. According to the most popular definition, *pharmaceutical analysis can be simply defined as analysis of a pharmaceutical compound or drugs*. This branch of analytical chemistry investigates new drugs, contaminants in pharmaceutical preparations,

the pharmacokinetics and metabolism of drugs as well as toxicological and biotechnological topics (1, 2). The term pharmaceutical analysis also refers to the analysis of plant raw materials which are used in the production of pharmaceutical preparations and herbal drugs (3).

Although many published studies deal with the present-day position of chromatographic techniques in pharmaceutical analysis (1, 4, 5), there are no relevant publications about the historical development of these techniques. At the same time, it is noteworthy that chromatographic techniques have been used in pharmaceutical analysis since the interwar period. The aim of this work is to provide information about the development of chromatographic techniques

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used in pharmaceutical analysis. The sources of information were Polish pharmaceutical journals, i.e., „Kronika Farmaceutyczna”, „Farmacja Współczesna”, „Wiadomości Farmaceutyczne”, „Acta Poloniae Pharmaceutica”, „Farmacja Polska”, „Annales UMCS sectio DDD Pharmacia” and „Dissertationes Pharmaceuticae” that were available at the library of the Jagiellonian University Medical College and the University of Warsaw Library.

Two information databases, namely, Polish Medical Bibliography (The Central Medical Library, Warsaw) and the PubMed database (US National Library of Medicine National Institutes of Health, Bethesda) were also searched.

Capillary analysis

In retrospect, there is no doubt that chromatographic techniques have played a key role in the development of research in the field of pharmaceutical analysis. The first application of chromatography to pharmaceutical analysis can be traced back to the use of a so-called *capillary analysis*. The principles of this technique were presented in two textbooks authored by Professor Bronisław Koskowski, a member of the Faculty of Pharmacy, University of Warsaw. In the textbook titled „The science of preparing drugs and their forms” (1925), lectures on research methodology using *capillary analysis* can be found. The aforementioned analysis was based on the mechanism described for paper chromatography, however, a typical mobile phase and the sample application spot were lacking. The process of chromatographic separation is a result of the flow of fluid sample through paper. The sample components move at different rates due to the differences in their affinity for the stationary phase. In the case of pharmaceutical analysis, the samples were mostly alcoholic or aqueous extracts of plant materials (6). In his second textbook, titled „Capillary analysis applied to assess the identity and goodness of pharmaceutical preparations” (1933), Koskowski presented the results of *capillary analysis* carried out for all tinctures that were supposed to be included in „Polish Pharmacopoeia II”. There were also colorful illustrations showing *capillary images* of the studied tinctures. All this information was supposed to serve as guidelines for routine testing performed in pharmaceutical laboratories (7).

In the 1930s, *capillary analysis* was used in several works dedicated to the qualitative evaluation of the composition of herbal tinctures. In 1932, Tadeusz Goettinger published the article titled „Detection of alkaloids in tinctures by capillary analysis” in „Wiadomości Farmaceutyczne” (8),

while Ludmiła Świerczyńska published a paper titled „About the possible applications of capillary analyses to study magistral preparations” in „Farmacja Współczesna”. Both publications were based on studies conducted in the Gessner Pharmacy laboratory in Warsaw (9). In 1937, capillary analysis was criticized by Władysław Karaffa-Korbutt, a professor of the Stefan Batory University in Vilnius, in the paper titled „Capillary-luminescent analysis of pharmaceutical preparations”. Karaffa-Korbutt pointed out the shortcomings of *capillary analysis* and, at the same time, proposed the modification, i.e., the use of quartz lamp for analyzing the *capillary images*. The modified technique produced more characteristic and reproducible results (10).

In the postwar period, *capillary analysis* was replaced by paper chromatography. However, several publications employing *capillary analysis* and dealing with the subject of qualitative differentiation of tinctures obtained from the medicinal plants growing in various locations, harvested at different times, or obtained from various plant organs (11, 12) were still published.

Column chromatography

The technique of classical column chromatography and the possibility of its use in pharmaceutical analysis were described in 1949 in a review article titled „The chromatographic analysis (chromatography) and its applications” and authored by Jan Muszyński, a professor at the Faculty of Pharmacy, Medical University of Lodz and President of the Polish Pharmaceutical Society. After a detailed presentation of the mechanism of column chromatography, the latest achievements in the field of chromatography were described in the next section of the aforementioned paper. A so-called *ultra-chromatography* technique using the analytical quartz lamp was among the presented methods. Thus, it was possible to study colorless compounds passing through a chromatographic column that were characterized by their fluorescence under UV irradiation. Based on the data from foreign literature, the author described several types of products that could be studied with this technique, i.e., leaf pigments, aniline dyes, alkaloid raw materials (analyzed *via ultra-chromatography*), and the separation of antimony and bismuth salts (13, 14).

In 1952, a methodological work titled „Chromatographic and biological studies of food dyes with particular emphasis on erythrosine” was published in „Acta Poloniae Pharmaceutica”. In this publication, 5 out of 30 tested food colorants were determined by column chromatography employing



different adsorbents, i.e., aluminum oxide, talc and calcium carbonate (15). It should be emphasized that the aforementioned study was also the first work employing a chromatographic technique that had been published in „Acta Poloniae Pharmaceutica”.

In later years, column chromatography was used in pharmaceutical analysis rarely, among others as a quantitative analysis technique. The results were presented as so-called *elution diagrams* (16). Column chromatography was also used as a preparative technique, as well as an auxiliary tool for a new chromatographic techniques, such as paper chromatography and thin layer chromatography (17-19). In the 1990s, column chromatography was used in the Immobilized Metal Ion Affinity Chromatography to study biomolecules, i.e., peptides and proteins (20).

Paper chromatography (PC)

The first Polish publication employing paper chromatography was dedicated to the qualitative and quantitative identification of medicinal substances. It was published in „Acta Poloniae Pharmaceutica” in 1952 under the title „The chromatographic separation of B vitamins”. The use of chromatographic techniques in this work was motivated by the small amount of test material needed. The analysis was conducted by using different mobile phase compositions, and an attempt was made to perform the quantitative analysis as well. A method of sample preparation and the analysis of yeast and bovine blood has been described (21). In 1953, a review article dedicated to chromatographic techniques was published in „Farmacja Polska”. It mainly concerned paper chromatography and its capabilities, i.e., simplicity and high sensitivity. The possibility of separation and determination of analytes from the group of compounds posing numerous analytical problems was highlighted. This concerned, among others, amino acids and sugars, and biological samples such as human body fluids (22).

Another experimental study employing paper chromatography was published in „Acta Poloniae Pharmaceutica” in 1955. The report contained the results of chromatographic separation of alkaloids (23). On the other hand, the Polish journal „Farmacja Polska” published for the first time a chromatography-based article titled „Sulfonamides, improved method of separating using paper chromatography” in 1954 (24).

In the second half of the 1950s, the articles on the use of experimentally determined paper chromatography systems for analysis of different groups of phytochemical substances were published. *Inter*

alia, the qualitative assessment of the content of flavones in tinctures prepared from various species of the genus *Hypericum* (25), alkaloids in different parts of the genus *Vinca* (26), and triterpene acids in a variety of herbal raw materials (27-29) was performed. Methodological works have also been published, e.g., „Methodological studies on the quantification of amino acids separated by chromatography on paper” (30) and „Chromatography of barbiturates” (31).

In 1960, a specific type of paper chromatography was described under the name *elatography*. The aim of this technique was to obtain chromatographic separation of products due to chemical reaction on the paper. It was the opposite of classical paper chromatography, wherein the analytes were separated from the mixture. In the *elatography* technique, the components of the mixture tested, reacted with the reagents which were in a solvent forming the mobile phase. The analysis was characterized by the formation of reaction products and the direct separation of components in a sequential order (32).

In the 1960s, paper chromatography was used to analyze, among others, the samples of raw materials of plant origin and pharmaceutical preparations derived therefrom (33-42) as well as in toxicological studies (43). PC was employed to assess the composition of excipients in pharmaceutical preparations (44, 45) and to evaluate the quality of both the medicinal products and magistral preparations (46-50). PC was also applied to analyze the plant protection products (51).

A loss of interest in paper chromatography, which occurred in the 1970s, was associated with the rapid development of modern planar technique, namely, thin layer chromatography. In the following years, paper chromatography was used sporadically (52-54), most frequently as a two-dimensional paper chromatography (17, 18, 55-57).

Thin layer chromatography (TLC)

In 1962, a review article titled „Thin-layer chromatography in the pharmaceutical analysis” was published in which TLC technique, named an *open column*, had been described as a sign of progress in the development of chromatographic techniques. The article emphasized that TLC combines the advantages of both column chromatography and paper chromatography as well as eliminates the disadvantages of each of these techniques. Detailed instructions were given on how to prepare the plates, and the method for plate analysis was described. The application notes related to TLC from foreign journals were also listed (58).



In 1963, two experimental works on employing TLC to investigate phytochemicals were published in „Farmacja Polska” and „Acta Poloniae Pharmaceutica” (59, 60). In the second half of the 1960s, the results of the analysis of alkaloid raw materials were primarily reported (61-65). Later, the attempts were made to apply TLC to study the stability of medications and medicinal substances (66-71) as well as to research the medicinal chemistry topics (72, 73). The articles dedicated to methodology (74) and new technical solutions (75, 76) were also published. The results of analysis conducted by using paper and thin layer chromatography (77, 78) were reported, while a comparative analysis of these results served as a basis for specifying the advantages of TLC, especially in terms of the significant reduction of the time of analysis (79).

In the 1970s, several scientific studies employing TLC were published annually in the Polish pharmaceutical journals. The authors of these works were not only the employees of pharmaceutical departments at the Polish medical institutions of higher education, but also the employees of the Institute of Medicines in Warsaw and control laboratories of the Board of Pharmacies. The published papers included the following topics: new chromatographic systems designed to investigate the properly prepared samples (80); determination of active compounds in pharmaceutical formulations (81-88); additives in drugs (89); and drug decomposition products (90). A number of studies concerning phytochemical research, including the research of herbal raw materials (91) and the assessment of the identity and quality of herbal medicines (92, 93), were also published.

In the 1980s and 1990s, the declining interest in thin layer chromatography was observed. Then, the most commonly used TLC method, was two-dimensional thin layer chromatography (57, 94-96). It was also used spectrophotometric technique to determine compounds previously isolated from TLC plates on which the chromatographic separation was carried out (97, 98). At the end of the 1970s, pharmaceutical laboratories started to use densitometry as a method of detection in TLC (99). The possibility of simultaneous qualitative and quantitative analysis resulted in an increase in the number of publications employing TLC as a method of investigation (100-102), for example, a few papers per year were published in „Acta Poloniae Pharmaceutica”. The number of such papers was similar to the number of publications in which the authors had used newer chromatographic techniques based on the mechanism of column chromatography, e.g., gas chromatography.

Gas chromatography (GC)

In 1962, a review article titled „Gas chromatography and its application” was published in „Farmacja Polska”. Gas chromatography was described as one of the greatest achievements of analytical chemistry in the post-war era (103). However, the first publication employing gas chromatography in experimental studies, titled „Study of the physico-chemical properties of oil from the seeds of *Xanthium orientale* L.”, was only printed in „Farmacja Polska” in 1968. Scientists from the Medical University of Gdansk reported on the application of „Chromatoprep” chromatograph, which had been constructed at the Faculty of Chemistry, Gdansk University of Technology (104).

Since then, articles on the application of gas chromatography in pharmaceutical research have been published regularly. In the late 1960s and 1970s, the publications primarily focused on the analysis of essential oils (105, 106). The variability of qualitative composition of oils in dependency on the plant harvest time (107, 108) and the content of pesticide residues were investigated (109). Many studies described the possibility of using gas chromatography to assess the composition of psychotropic drugs (110), and to determine the degradation products of medicinal substances in selected dosage forms (111, 112).

Since the 1980s, gas chromatography has become a leading tool for analyzing psychotropic drugs and narcotics in human blood (113-115). The determination of contaminants in herbal raw materials and the dosage forms obtained from these materials was also conducted (116, 117), as well as phytochemical researches (118). In 1990s, a mass spectrometer has become the most widely used detector, which enabled the use of gas chromatography in medicinal chemistry (119, 120). At present, gas chromatography is still in use, which results in some articles being published annually in „Acta Poloniae Pharmaceutica”. However, in terms of the number of publications, GC is inferior to the youngest chromatographic technique used in pharmaceutical analysis, namely, high performance liquid chromatography.

High performance liquid chromatography (HPLC)

In 1979, a review article concerning high pressure (performance) liquid chromatography was published in „Farmacja Polska” under the title „Chemically bonded stationary phases, and their use in pharmaceutical analysis by high performance liquid chromatography” (121). Two years later, the first paper



on experimental research, titled „High performance liquid chromatography (HPLC) analysis of piracetam next to its degradation products” (122), was published.

Initially, there were relatively few publications employing high performance liquid chromatography, which dealt with either the degradation products and impurities in pharmaceutical formulations (123-127), or the samples of human bodily fluids and biologically important molecules (128-131). HPLC was a comparative method, both for research using spectroscopic methods (132), as well as for an older chromatographic techniques, especially TLC (133, 134). High performance liquid chromatography was also used for quantitative analysis, as a complement to qualitative tests performed by TLC (135).

In the early days of the HPLC technique, analysts personally constructed chromatographs. An example can be a device created in the Department of Inorganic Chemistry at the Medical University of Lublin (136).

The rapid development in the field of practical application of HPLC took place only in the first decade of the 21st century. Since then, several articles employing this technique have been published each year in „Acta Poloniae Pharmaceutica”. The advanced methods of sample preparation and a wide range of detectors used in HPLC-based procedures allowed for studying chemical compounds with various structures, including active substances in plant material, dosage forms, and biological material.

DISCUSSION AND CONCLUSIONS

Nowadays, chromatographic techniques are considered the most popular analytical methods in

pharmaceutical analysis (1, 2, 137). High performance liquid chromatography has become, in a relatively short time, the most commonly used chromatographic technique in pharmaceutical research. The coupling of constantly perfected and developed detection techniques to HPLC creates analytical methods that enable the investigations of the largest possible number of chemical compounds (4, 5, 137, 138). The methods of sample preparation prior to analysis are a critical factor in the application of high performance liquid chromatography to pharmaceutical analysis. The sample preparation allows the purification of the collected material, the enrichment of analytes, and the analysis of samples with complex matrices in which the analytes are present at trace level concentrations. This applies particularly well to the samples of biological material collected from a patient (1, 138, 139). The testimony to the rapidly growing popularity of HPLC is the number of monographs suggesting the use of this technique that can be found in the contemporary Polish pharmacopoeia. For example, „Polish Pharmacopoeia VI” (2002) (140) recommends the application of high performance liquid chromatography in 124 monographs, while this method was generally not considered in the previous „Polish Pharmacopoeia V” (1990) (141).

Gas chromatography is also used in pharmaceutical analysis, however, due to its limitations, it has never become a dominant tool. Moreover, its application is limited to the selected groups of chemical compounds (138, 142). The planar methods, such as *capillary analysis* and paper chromatography, should be considered historical techniques (143). Despite the fact that „Polish Pharmacopoeia IV” from 1965 contained 12 monographs recommending these analytical methods (144), the contemporary pharmacopoeia does not contain any.

Thin layer chromatography is a unique planar technique. For the half of a century, it has maintained a strong position, especially in the analysis of raw materials and herbal medicines. Although TLC is in many ways inferior to high performance liquid chromatography, it still provides rapid and simple testing (1, 3, 145). The popularity of thin layer chromatography in pharmaceutical analysis is reflected by the increasing number of pharmacopoeial monographs, ranging from 184 in „Polish Pharmacopoeia V” (141) to 558 in „Polish Pharmacopoeia VI” (140).

The aforementioned interdependencies between different chromatographic techniques can be characterized by the number of papers published

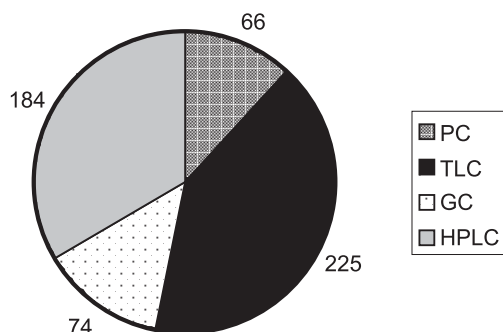


Figure 1. The number of publications describing the possibilities of using chromatographic techniques (based on articles published in the years 1952-2014 in „Acta Poloniae Pharmaceutica”, according to the PubMed database). PC - paper chromatography, TLC - thin layer chromatography, GC - gas chromatography, HPLC - high performance liquid chromatography



in pharmaceuticals journals. The first two publications dealing with the use of column and paper chromatography in experimental studies were published in „Acta Poloniae Pharmaceutica” in 1952. By the end of 2014, 557 publications employing chromatographic techniques and published in „Acta Poloniae Pharmaceutica” were registered in the PubMed database (Fig. 1). In 225 of these publications, thin layer chromatography was used. The aforementioned studies were published over the period of 52 years. On the other hand, at the end of 2014, the number of works employing high performance liquid chromatography was 184. However, they were published over the period of 30 years.

Currently, high performance liquid chromatography is the most popular and fastest growing chromatographic technique in the field of pharmaceutical analysis. Thin layer chromatography is still popular, but HPLC has surpassed it in terms of the number of publications published per year. On the other hand, gas chromatography has a stable position in pharmaceutical analysis. However, the limitations of this technique result in the publication of only a few studies employing GC per year. In contrast, paper chromatography can only be considered a technique of the past. It should be emphasized, however, that this technique was used for the longest time as indicated by the relevant articles published in the journal „Acta Poloniae Pharmaceutica”.

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