Archives of Environmental Protection
Vol. 45 no. 1 pp. 42–49

PL ISSN 2083-4772
DOI 10.24425/aep.2019.124829

The use of gas chromatography for determining pharmaceutical residues in clinical, cosmetic, food and environmental samples in the light of the requirements of sustainable development

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Keywords: pharmaceutical residues determination, GC-based methods, sustainable development, competitive advantage, Polish market.

Abstract: The sustainable development of human activities is directly related to the protection of the environment by lowering the anthropogenic stress. Pharmaceuticals – due to their growing consumption (use in medicine, veterinary, animal production, cosmetics) and their incomplete removal in wastewater treatment plants – are classified as a group of new and rapidly emerging pollutants which have been proven to have a negative impact onto water organisms. In order to ensure the proper protection of human health and the environment there is an urgent necessity of determining pharmaceuticals in clinical, cosmetic, food and environmental samples. Gas (GC) and high performance liquid chromatography (HPLC) are valuable techniques for such determination, especially when they are coupled with mass spectrometry (GC-MS; LC-MS) or tandem mass spectrometry (GC-MS/MS; LC-MS/MS). The purpose of this paper is to present an analysis of sustainability features of analytical techniques in the light of necessity to determine trace amounts of pharmaceuticals in the aforementioned different matrices. Using the Delphi method we performed an analysis of the key sources of the competitive advantages of the application of GC and GC-MS techniques for determining the pharmaceutical residue in clinical, cosmetic, food and environmental samples – compared to techniques based on HPLC or LC-MS. The analysis covered the following areas: (i) the features of the technique, (ii) the price, and (iii) the applicability in various sectors of economy.

Introduction

The necessity for determining a broad spectrum of pharmaceuticals in different matrices requires laboratories to have various measurement systems at disposal because the possibility of conducting measurements using different detection systems can increase efficiency and profitability of the analyses performed. We identified the main benefits that can be obtained by individual entities as a result of implementing GC / GC-MS techniques for such analyses. When examining the sources of the competitive advantage, we used the Delphi method (Hallowell and Gambatese 2010) where the leading Polish specialists using GC and GC-MS for determination of pharmaceutical residues in the different matrices participated in the study. The idea of Delphi as an example of a heuristic method is to design a questionnaire referring to the studied scientific problem and then distribute it to the leading experts in a certain field. The questionnaires are delivered to the selected number of experts in a number of rounds. A procedure designed and implemented in this way allows the researchers as Hallowell and Gambatese (2010) underline to obtain information of a high reliability. The Delphi method is also used by researchers to evaluate risks related to a certain area (del Caño and de la Cruz 2002).

We implemented the research procedure in the following steps: 1. design of the questionnaire intended to be used in the study, 2. conducting a pilot study by sending the questionnaire to two randomly selected experts asking for comments whether the questionnaire was properly prepared and contained questions which were clear to the respondent, 3. identification of the leading experts working with the chromatographic techniques according to the objective of the study, 4. selection of the most reliable experts, 5. distribution of the questionnaires, 6. study of the obtained responses and identification of the fields of consensus and conflict, 7. repeated analysis of the questionnaires to identify the strengths and weakness of the GC, GC-MS in comparison to other chromatographic techniques.

Key sources of the competitive advantage of gas chromatography

In the beginning it has to be underlined that the possibility of using GC and GC-MS is available only for 35% of the pharmaceuticals existing either in the native form (5%) or

as volatile derivatives (30%) (own calculation). For the remaining 65% of the pharmaceuticals, the technique used most frequently is HPLC or LC-MS/MS. Table 1 summarizes the most important attributes in favor of using the GC and the GC-MS techniques compared to LC-based techniques.

Gas chromatography is classified as a high-resolution technique. This is mainly due to the many times higher efficiency of GC columns, in relation to columns used in LC. The large number of the analytes possible to be determined in one chromatographic run directly influences the reduction of

Table 1. Analysis of the key sources of the competitive advantage of GC compared to LC techniques, in relation to its attributes

No.	The source of competitive advantage	Rating of the advantage	Characteristic
1	Uniqueness of the service	Significant for 35% of pharmaceuticals	The existing demand for determination of pharmaceuticals using GC is not large. Literature data also indicates a dominant share of the LC technique used in such analyses, e.g.: according to Scopus, as of 30.01.2018, 3874 reports on the analyses of pharmaceutical residues using the GC technique and respectively 18137 reports using the LC technique were recorded. It should be underlined, that when determinations must be carried out in regulated fields, the selection of the measuring technique is regulated by the legal requirements. In other areas, meteorological requirements, availability of equipment and personnel qualifications are decisive. Summing up, few companies identify residuals of pharmaceuticals using GC and GC-MS, hence the uniqueness of the availability of the service for 35% of the pharmaceuticals can be significant.
2	Duration of the analysis	Average	Time of chromatographic 'run' depends on the number of analytes taken as targets and MS abilities. When comparing the GC and LC analysis of for example 20 pharmaceuticals, the time of analysis is very comparable. This means that the advantage of the GC and the GC-MS techniques in this area is not significant.
3	Availability of the technique	Average	High availability of GC and GC-MS as well as of HPLC and LC-MS equipment in the market and in laboratories causes the prevalence of the GC-based techniques in this area to be insignificant.
4	Accuracy of the result	Significant	High accuracy of the obtained result is attributable to a far lower matrix effects affecting the results of final determinations, in comparison to the analyses using LC-MS (Caban et al. 2012). It is associated with the technique ion source used. Electron Ionization (EI, in case of GC-MS) is less sensitive to matrix effects compared to Electro-Spray Ionization (ESI) (used routinely in LC-MS) (Biswas and Mitra 2013). It is important because certified reference materials (Certified Reference Materials – CRMs) of adequate quality are practically unavailable for a large part of the analyses and matrices, therefore having patterns of appropriate quality as well as an adequately optimized methodology is crucial for obtaining a highly accurate result. The examples of negative matrix impact onto chromatographic analysis can be found in literature (Caban et al. 2012, Garrido et al. 2009). In general, the GC-MS maintained in clear conditions are much less affected by matrix effects, compared to LC-MS. This results in more accurate and reliable results.
5	Detection limits	Average	Sensitivity of the GC-MS technique is comparable to the LC-MS. It is also sufficient in terms of the expected necessary concentration ranges (ng/l or ng/kg), however, application of SPE-GC is associated with a higher concentration ratio of the sample, e.g. transfer of the analytes from a 500 ml sample to a 0.1 ml volume (the volume of the derivatising agent) gives a concentration factor of 5000 (Caban et al. 2016); for this type of analyses SPE-LC-MS most commonly is 500. An advantage of GC and GC-MS in this area, however, is not significant and obtained pharmaceuticals detection limits derive mostly from extraction techniques applied (Liška and Slobodník 1996).
6	Waste production	Significant	Low waste production, in comparison to the LC-MS technique, is associated with application of a carrier gas as a mobile phase. Such method additionally is much cheaper and environmentally friendly, because there is no need for disposal of the solvent wastes. Consumption of solvents in the techniques of HPLC nowadays can be limited up to 90% (Caban et al. 2015), however, the cost of such solutions is many times higher. GC is often so-called green technique in chromatography (Biswas and Mitra 2013).
7	The rate of implementation in routine analyses	Significant	Much faster to be implemented for routine analyses in environmental and biomedical laboratories, in comparison with LC-MS-based methods. It results from the lower cost (see Table 2). In addition, the time needed to train a worker to operate GC-MS equipment is shorter due to easier optimization of this system's operation.

Source: own elaboration

the price of obtaining a single piece of analytical information. Although the resolution is much better in chromatographic part of GC-MS than in LC-MS, it is difficult to talk about competitiveness, since in typical target analyses, such as determining pharmaceutical residues, determination of analytes is based on mass spectrometric detection e.g. on monitoring a selected ion-fragmentation (SIM) or on multiple fragmentation (multiple reaction monitoring mode – MRM) instead of chromatographic parameters. For this reason, nowadays the overlap of analyte signals during chromatographic separation is no longer a big problem. Higher resolution of GC has become, however, a definite advantage when much simpler detectors are used instead of a mass spectrometer.

The GC and the GC-MS techniques are characterized by very small, in comparison to HPLC and LC-MS, amount of waste generated during analyses. The consumption of carrier gases in relation to the consumption of organic liquid phases is also many times cheaper and environmentally friendly. Although solvent consumption in liquid chromatography presently is limited due to introduction of ultra-performance systems (Caban et al. 2012) allowing for the reduction of the amount of the mobile phase even by 90%, the cost of such solutions is many times higher.

In 2015, we conducted a study using a diagnostic survey method, which encompassed 277 Polish institutions and other entities employing in their activities analytical methods (Sadkowska et al. 2017). The aforementioned institutions included first of all selected laboratories, research institutes as well as the laboratories operating within the structures of higher education. Although chromatographic techniques were used by a relatively high number of the studied institutions and enterprises (private and public), the analyses confirmed that determination of the residues of pharmaceuticals in environmental, food and clinical samples is performed by a small group of entities (a total of 62 entities). 7 entities confirmed to have analyzed the residues of pharmaceuticals in environmental samples, 13 entities in food samples, 12 entities in clinical samples, and 30 entities in other matrices.

The studied entities indicated that their decision to select GC-MS technique instead of LC-MS was driven, among other factors, by the cost of equipment. The other group of respondents confirmed that they preferred to use the services of external laboratories because in the case of performing not too many analyses, it was cheaper than purchasing and operating GC-MS / LC-MS equipment. It was also interesting to observe that GC as an analytical techniques were used mainly by the veterinary inspectorates as well as institutions controlling water and food quality.

The key sources of the competitive advantage of GC and GC-MS in terms of the price

In many cases, the decisive factor in applying a given solution by an analytical unit is its total cost, calculated as the sum of expenditures that are necessary to purchase the equipment as well as the sum of operating costs (services, consumables, energy etc.). The strengths characterizing GC and GC-MS in respect to this area are presented in Table 2.

In the case of GC and GC-MS, the "value chain" is formed, primarily by a lower, compared to LC, purchase cost of equipment, a lower cost of basic utilities such as electricity and water, and, in consequence, greater opportunities for performing analyses at a competitive price. Additionally,

Table 2. The key sources of the competitive advantage of GC in terms of the price compared to LC

No.	The source of competitive advantage	Rating of the advantage	Characteristic
1	The cost of operation / maintenance	Significant	Lower compared to LC-MS. Such level of the costs of analyses is result--associated with lower waste production, and thus with lower utility costs (water and electricity), lower costs of the chemical agents and the accessories necessary for analysis performance (organic solvents, salts, columns, gaskets, filters).
2	The purchase costs of a chromatograph	Significant	Average expenditures necessary to purchase the equipment for performing GC-MS analyses are 60 000 euro, when purchasing LC-MS equipment the cost is around 375 000 euro. The life cycle of the apparatus, in both cases, is comparable, with an average of 10 years. The amount of the expenditures needed for purchasing equipment directly affects the amount of the depreciation, which, consequently, affects the cost of a single analysis.
3	The cost of waste disposal	Significant	Lower, compared to LC-MS methods. The mobile phase in the GC and the GC-MS techniques is a neutral gas, while HPLC and the LC-MS techniques produced a large volumes of liquids hardly to regenerate and with toxic properties (organic solvents).
4	The total cost of analysis	Significant	The cost an analogous test using GC-MS is about 40–50 euro (Polish market) per a sample, using LC-MS about 50–80 euro respectively.
5	The personnel work cost	Relative	The GC-MS equipment is easier in use, optimize and servicing compared to LC-MS. Thereby the comparably or less qualified and experienced person can work on GC-MS. As a result the estimated costs of employing this personnel can be lower compared to LC-MS.

Source: own elaboration

as mentioned before, a smaller amount of waste plays an important role. The costs of equipment servicing, in the case of both chromatographic techniques, are comparable.

The key sources of the competitive advantage of GC and GC-MS in terms of its possible applications

Analyses aimed at determination of residuals of pharmaceuticals in environmental, clinical, cosmetic and food samples have been and will continue to be performed increasingly, so as to meet the needs of the pharmaceutical industry and for the purpose of chemical synthesis (evaluation of the composition and the purity of products), forensics and anti-doping control (search for banned chemical compounds in blood and in other biological traces), environmental protection and work--environment sector (control of air, water and soil purity and quality), and food industry (analyzing the composition, purity and adulteration of food products) (Table 3). Regardless of the type of sector, the laboratories using chromatographic measurements face increasingly higher demands regarding the quality of the services provided and the solutions offered. The increase in the above requirements derives from both an increased competitiveness on the analytical market and a transformation of the legislative regulations related to the increasing influence of international guidelines, including appropriate European Union directives. For instance, according to the *European Commission Decision 2002/657/EC*, both gas chromatography and liquid chromatography, especially in combination with mass spectrometry, have to meet the criteria set for the methods confirming presence of pharmaceuticals in food samples.

The potential of gas chromatography technique is considerably associated with the impact of the legislative conditions. It mainly concerns the anticipated national legal acts and European Union regulations (in the form of appropriate directives), which will impose an obligation to test for residuals of pharmaceuticals in the environment, *inter alia*, in surface waters and in drinking water. Such legislative regulations exist for food products.

Table 3. The key sources of the competitive advantage of GC and GC-MS in terms of its possible applications

No.	The source of competitive advantage	Rating of the advantage	Characteristic
1	The potential for broader application	Average	GC can be widely used fin the following sectors: or the food, the pharmaceutical, the cosmetic, the anti-doping, the criminal, the natural environment.
2	The gaps on the chemical analysis market	Significant	On the Polish market, there is a small number of entities which provide pharmaceutical residue determination services using GC and GC-MS. These entities include, among others: the Institute of Environmental Protection (Instytut Ochrony Srodowiska), the National Veterinary Institute (Panstwowy Instytut Weterynaryjny) and the Forensic Institute (Instytut Ekspertyz Sądowych). There is no knowledge on, firstly – the possibilities offered by application of the gas chromatography techniques, secondly – on using the above techniques for determination of pharmaceutical residues in particular matrices.
3	The macroeconomic conditions	Average	The government's intention to ensure maximum safety in terms of the use of pharmaceuticals as well as to minimize such side effects as environmental pollution. This is manifested by, inter alia, the legislative solutions compatible with European Union requirements.
4	Environmental conditions	Significant	Related to the activities aimed at maximum protection of the environment from the possible negative effects of the presence of pharmaceuticals in it. Literature reports provide numerous examples of the disturbance of proper functioning of aquatic and terrestrial organisms caused by pharmaceutical residues (Aurélien et al. 2013, Fent et al. 2006). In Poland, such studies are conducted occasionally, despite the fact that Poland is one of the world leaders in terms of consumption of pharmaceuticals (Compare Sadkowska et al. 2017)
5	Legislative conditions	Average	Documents introducing recommendations for research involving evaluation of the degree of environmental pollution caused by pharmaceutical residues (a) as well as those associated with the need to use analytical methods of adequate quality (b): a) A Report of the Commission for the European Union Parliament and Council, on the outcome of the review of Annex X of the European Union Parliament and Council Directive 2000/60/EC on priority substances in the field of water policy dated 31.01.2012. b) Decision of the Commission 2002/657/EC dated 14.08.2002, implementing the Council Directive 96/23/EC on the results of analytical methods and their interpretation.

Source: own elaboration.

The use of GC technique is, unfortunately, connected with the reduction of analytes which can be determined in a given matrix. Most pharmaceuticals are polar, therefore, direct analyses using GC are not possible. In many cases, the solution is to perform a derivatization (a chemical reaction aimed at increasing the volatility and thermal stability of an analyte) (Caban et al. 2014). Currently, this process does not pose a major problem – the agents applied are becoming cheaper and safer, while the uncomplicated procedures of the conduct can extend the analysis by mere 5–30 minutes (Caban et al. 2012). As was mentioned, only 5% can be directly analyzed using GC (e.g. selected anti-depressant medications), while about 30% after derivatization. The pharmaceuticals which are easily derivatized are analgesics, beta-blockers and beta--agonists or steroid hormones (Caban et al. 2014, Caban et al. 2011). Antibiotics and antiparasitic pharmaceuticals, which are important from the perspective of quality control of food products, cannot be determined by GC techniques. Utility of GC and GC-MS is thus linked to a quite large reduction of the number of the pharmaceuticals which can be monitored – HPLC and LC-MS techniques are, in this respect, more universal. The greatest possibilities of using GC-MS technique lie in the environmental protection sector, in connection with the planned introduction of the need to monitor diclofenac and estrogenic hormones in surface waters. The procedures for determining pharmaceuticals using SPE-GC-MS are characterized by appropriate sensitivity and selectiveness and are available in the literature (Caban et al. 2011, Daughton 2016). What is more, GC techniques are adequate to determine several pharmaceuticals degradation/transformation products together with native analytes (Aznar et al. 2014, Rabiet et al. 2006) belonging to different therapeutic classes (anti-inflammatory/analgesics, lipid regulators, antiepileptics, β-blockers and antidepressants.

Another factor which can be important for users is sensitivity (limits of detections LOD and limits of quantification LOQ) of techniques applied for the detection of pharmaceuticals. This aspect is important rather for the samples in which the analytes were obtained in very low concentration, for example, environmental waters. Current chromatographic techniques coupled with mass spectrometry have generally the same potential of detection. All the detection limits ranged in ng/L levels. For example, the detection limit of diclofenac (NSAID) ranged between the 60 ng/L (López-Serna et al. 2012) and 0.02 ng/L (Loos et al. 2009) for environmental waters. For this pharmaceutical and for carbamazepine, lower LOD was obtained for methods based on GC-MS (Caban et al. 2016), but it was dependent on extraction techniques used for water extraction and sample volume (Białk-Bielińska et al. 2016). The low detection limits are driven by the detector use; thereby the LC-MS and GC-MS techniques have the same potential. Factors such as linearity, accuracy and precision presented in the literature data are also comparable, because the validation process forces certain limits of these parameters (Hess et al. 2018, Hou et al. 2015, Petrie et al. 2014, Riemenschneider et al. 2017, Sousa et al. 2018, Togola and Budzinski 2008, Vazquez-Roig et al. 2010).

It is also worth noting that in the national Marine Water Monitoring for the years 2014–2019 (created in accordance with the requirements of the Marine Strategy Framework Directive on 2008/56/EC), one of the core indications recommended by the Commission Decision 2010/477/EC dated 01 September 2010 (*Commission Decision of 1 September 2010 on the Criteria and the Methodological Standards on Good Environmental Status of Marine Waters*) and agreed upon as part of the work of the HELCOM CORESET (http://www.helcom.fi/helcom-at-work/projects/completed-projects/coreset) group, included two pharmaceuticals – diclofenac and 17-alpha-ethinylestradiol, for which GC-MS is the reference technique.

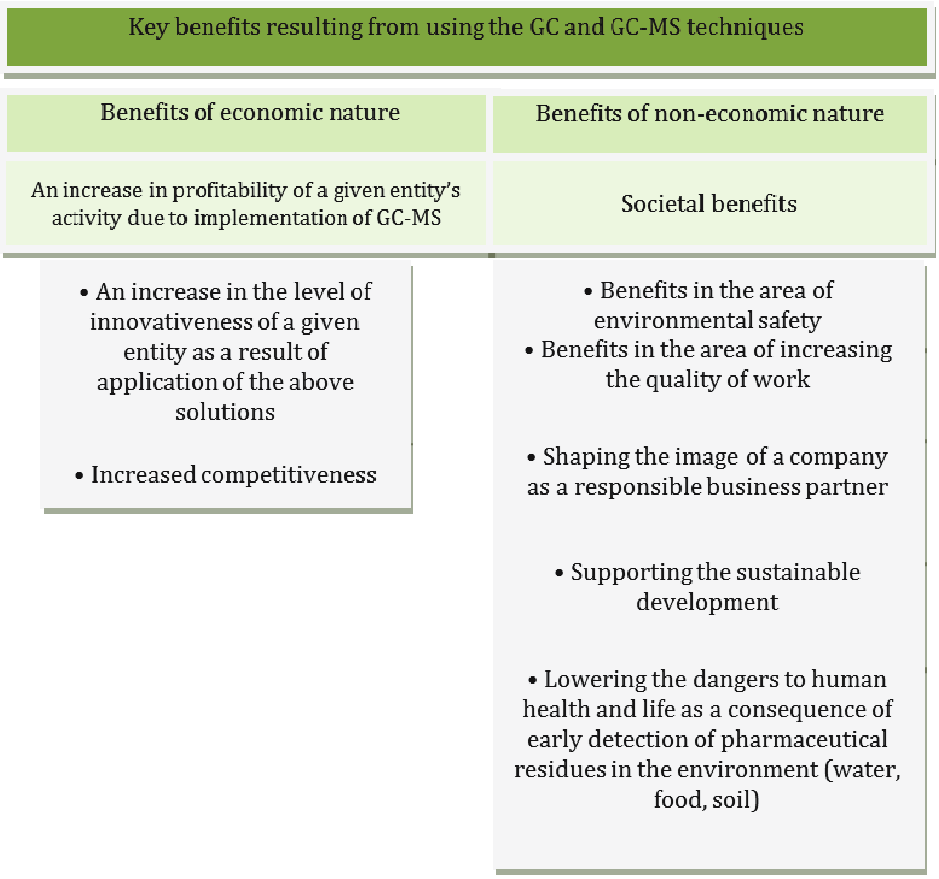
Potential benefits of using the GC and the GC-MS techniques in the light of the demands of the sustainable development

The set of competitive advantages characterizing GC and GC-MS as tools for determining pharmaceutical residues translates into potential benefits that can be gained by enterprises, scientific units, research laboratories and other entities as a result of using these techniques (Figure 1). Consequently, it is reflected by financial results achieved by the entities which choose to use this particular technique in their activity (Jóźwiakowski et al. 2015).

In the case of the benefits of economic nature, the possibility to improve profitability of the entity using GC and GC-MS is particularly important, in addition to increasing its innovativeness. Profitability is understood as the ratio of the profit generated by a given entity to the examined parameter, for instance, to the level of the sales. Improvement of profitability mainly results from the lower cost of purchasing the equipment used for GC, lower utility costs and a lower level of wasting, which consequently affect the cost of a single test. At the same time, it should be noted that reduction of the costs of conducted activity can serve as the basis for reducing the price of the services involving analysis of pharmaceutical residue content in the analyzed material. It can, in consequence, mean the possibility of "entering" new areas of use that have not been available due to the high cost of the performed analyses. Currently, GC and LC techniques enable determining pharmaceutical residues in a growing number of different matrices (Biswas and Mitra 2013, Daughton 2016, Görög 2011, World Health Organization 2011, Mohamed 2015, Petrović et al. 2005, Puckowski et al. 2016, Sadkowska et al. 2017, Santos et al. 2010, Tadeo et al. 2012) a review of the liquid chromatography-tandem mass spectrometry (LC-MS/MS, however, these techniques have never been studied in the light of the potential influence they might have in the process of supporting the sustainable development.

Conclusions

Gas chromatography techniques allow for the achievement of tangible benefits both in the economic and non-economic sense, mainly related to its significant, positive impact on the environment in comparison to LC. GC is a relatively cheap analytical technique, available for a growing number of entities, both those conducting research as well as the recipients of research services (lower cost of long-term research should reduce the price of a single test). As a result it can facilitate compliance with the legal requirements related to the assessment of environmental risks (Generowicz and Iwanejko 2017, Załęska-Radziwiłł et al. 2011) is economically



Source: own elaboration.

Fig. 1. Identification of the key benefits associated with application of GC and GC-MS for pharmaceutical residues analysis

efficient, socially acceptable, with no negative effect to the natural environment and takes into account economy of all waste streams, including electrical and electronic waste (E-waste) and the manner of management of chemicals. It should be underlined, however, that in the case when a wide range of pharmaceuticals must be analyzed, there is need for maintaining various measurement systems by laboratories. It is expected that in the years to come, there will be an expansion of knowledge and of public awareness regarding the phenomenon of 'entrance of pharmaceuticals into the environment', leading to increase of the safety of products offered by pharmaceutical, cosmetic and food industries and decrease of the negative impact of these compounds onto the environment and human health.

Acknowledgments

The project has been financially supported by the Polish National Centre for Research and Development (Narodowe Centrum Badan i Rozwoju) within the grant number TANGO1/268806/NCBR/2015.

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Zastosowanie chromatografii gazowej do oznaczania pozostałości farmaceutyków w próbkach klinicznych, kosmetycznych, żywieniowych i środowiskowych w świetle wymagań zrównoważonego rozwoju

Streszczenie: Rozwój zrównoważony jest bezpośrednio związany z ochroną środowiska, w tym z obniżeniem stresu antropogenicznego. W związku z rosnącym zużyciem farmaceutyków w wielu sektorach gospodarki, w tym między innymi w medycynie, weterynarii, sektorze farmaceutycznym, kosmetycznym oraz ich niepełnym usuwaniem przez oczyszczalnie ścieków, pozostałości farmaceutyków docierają do środowiska, gdzie mogą oddziaływać na organizmy tam bytujące. W celu zapewnienia właściwej ochrony zdrowia ludzkiego oraz środowiska niezbędnym jest oznaczanie pozostałości farmaceutyków w próbkach klinicznych, kosmetycznych, żywieniowych oraz środowiskowych. Techniki oparte na chromatografii takie jak: chromatografia gazowa (*GC – Gas Chromatography*), wysokosprawna chromatografia cieczowa (*HPLC – High Performance Liquid Chromatography)* są szczególnie przydatne w oznaczaniu farmaceutyków w szczególności, kiedy techniki te są sprzężone ze spektrometrią mas (GC-MS, LC-MS) lub tandemową spektrometrią mas (GC-MS/MS; LC-MS/MS). Celem niniejszego artykułu jest analiza przewag konkurencyjnych techniki, jaką jest chromatografia gazowa w kontekście wymagań zrównoważonego rozwoju. Wykorzystując metodę delficką przenalizowano użyteczność i przewagi chromatografii gazowej w oznaczaniu pozostałości farmaceutyków w różnych próbkach – w porównaniu do technik opartych na HPLC oraz HPLC-MS. Na podstawie przeprowadzonych badań na rynku polskim, którymi objęto 277 podmiotów wykorzystujących w swojej działalności techniki analityczne, przedstawiono możliwości zastosowania techniki GC w poszczególnych sektorach gospodarki.