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DEVELOPMENT OF A METHOD FOR DETERMINING THE MORPHOLINIUM THIAZOTATE USING MORE ECONOMIC AND GREEN GC/MS ASSAY WITH AN FID DETECTOR

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One of the main steps in the pharmaceutical development of drugs is the choice of quality control methods. The correctness of the method must be confirmed by validation. In addition, manufacturers take into account various economic and environmental factors. It is especially important to determine the above aspects for domestic and promising drugs, such as the morpholinium thiazotate.

The aim. *During the development of methods for the routine analysis of medicinal products, attention should be paid to efficiency of analysis, budget, as well as their impact to the environment. Because of this reason, not only new methods for routine analysis should be developed. It is important this methods must be environmentally-friendly and cost-efficient.*

Materials and methods. *The determination of the morpholinium thiazotate was carried out by HPLC using the SunFire C18 (150×4.6 mm, 5.0 µm) and gas chromatography with a flame ionization detector using the Rxi-5 ms (30 m long, 0.25 mm outer diameter and 0.25 µm liquid stationary phase thickness).*

Results. *Various chromatographic methods for the routine quantitative analysis of morpholinium thiazotate were developed. The most suitable conditions for sample preparation were established. Proposed methods were compared to find the most ecological and economic.*

Conclusions. *Proposed methods were accurate and reliable. However, an environmental impact assessment showed that GC-FID is a more environmentally friendly and economical method of analysis. Using 12 Principles of green analytical chemistry, the overall "analytical GREEnness (AGREE)" scale for the proposed analytical approach was computed 0.72, showing the good greener nature of the proposed analytical approach*

Keywords: *HPLC, GC-FID, method development, analytical eco-scale, cost analysis, environmental, AGREE scale*

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1. Introduction

The production of innovative drugs is associated with the need to control the release of residues into the environment of potential APIs (active pharmaceutical ingredients) on an industrial scale. The drug development in the environment is carried out by chromatographic methods of analysis. Therefore, it is very important for the pharmaceutical industry that analysis should be an economical and environmentally friendly as much as possible.

Currently green analytical chemistry (GAC) is used. Green analytical chemistry is an aspect of the concept of green chemistry and sustainability. The GAC places particular emphasis on reducing the environmental impact of analytical procedures. There are several approaches to achieve this goal: pre-treatment of green samples [1], use of environmentally friendly solvents and reagents [2], reduction of the impact of chromatographic analysis by reducing the time of chromatographic separation [3], minimization of analytical devices [4].

Direct analytical methods (i.e., without sample preparation) are particularly preferred from point of view of GAC. Most analyses of organic compounds are performed using gas or liquid chromatography. Thus, it is very important that these methods have a negligible impact on the environment.

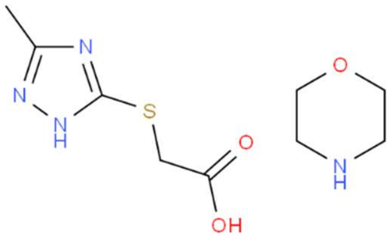

Fig. 1. Morpholinium thiazotate

Morpholinium thiazotate (Fig. 1) is the first native Ukrainian original drug. Now morpholinium thiazotate and its dosage forms are produced by pharmaceutical manufacturers that have the appropriate licenses and certificates of compliance with GMP conditions: SE "Plant of Chemical Reagents", NTK "Institute of Single Crystals" of the National Academy of Sciences of Ukraine (substance), corporation "Arterium" (2.5 % solution for injection and tablets), Pilot Plant GTsNLS LLC (eye drops), PJSC "Lekhim-Kharkiv" (suppositories) [4].

Preclinical studies performed in accordance with the requirements of the State Expert Center of the Ministry of Health of Ukraine showed that thiazotate morpholine exhibits high antioxidant, anti-ischemic, cardioprotective and antihypoxic properties.

The medicine is produced and used by the population in large quantities. Therefore, there is currently a need to develop an improved method of determination, which in the future will be used to determine morpholinium thiazotate in the environment.

The aim of this study is to develop and validate different chromatographic methods (HPLC, GC-FID) for the assessment of the morpholinium thiazotate and choose among them a less costly, more effective, and green technology.

The green data of the present analytical approach was assessed utilizing "AGREE: The Analytical Greenness Calculator".

2. Planning (methodology) of the research

The main stages of the study are:

– substantiation of the choice as the object of study of the morpholine thiazolate,

– study of previously developed methods of marriage;

– development of new methods of extraction and their improvement;

– conducting economic analysis and determining a more environmentally friendly method.

The planning of the experiment is implemented by assessing the risk analysis to achieve the goal, calculations, AGREE scale.

3. Materials and methods

3. 1. Reagents

Ultrapure water was obtained in the laboratory using a Milli-Q water purification system (Millipore, Billerica, MA, USA). Sodium perchlorate Sigma–Aldrich (St. Louis, MO, USA), acetonitrile (St. Louis, MO, USA). N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) (>99 %), N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) (>98.5 %) tetrahydrofuran, acetonitrile was purchased from Sigma–Aldrich (St. Louis, MO, USA). The GC-equipment was run with helium (purity 5.0) as the carrier gas was purchased from Gazchema (Lithuania). Standard sample of morpholinium thiazotate (PJSC "Halychpharm", Ukraine).

3. 2. Instrument

The study was performed on a chromatograph with a diode-array detector SPD-M20A (Shimadzu Company, Japan), pump LC-20AD (Shimadzu Company, Japan), column thermostat CTO20AC (Shimadzu Company, Japan) and automatic sample dispenser SIL-20A (Shimadzu Company, Japan), column SunFire C18, size or 150×4.6 mm, or 150×3.9 mm with a particle size of 5 µm or similar quality (Waters company, USA). Analyses were performed using a GC-2010 Plus Shimadzu with flame ionization detector and autosampler AOC-20i+s (Shimadzu Technologies, Kyoto, Japan). The separation of analytes was carried out on a with Rxi-5 ms (Restek Corporation, Bellefonte, PA, USA, capillary column (30 m long, 0.25 mm outer diameter and 0.25 µm liquid stationary phase thickness).

3. 3. Methods (chromatographic conditions, preparation of standards and samples)

3. 3. 1. Chromatographic conditions of HPLC method

Chromatography is performed on a liquid chromatograph with a spectrophotometric or diode-matrix detector under the following conditions: column SunFire C18, size or 150×4.6 mm, with a particle size of 5 µm or similar quality; the mobile phase was acetonitrile-water (25:75, v/v). This mobile phase was filtered through a 0.45 µm membrane filter (Millipore). Morpholinium thiazotate were quantified by DAD following RP-HPLC separation at 210 nm for morpholinium tiazotate. Flow rate and injection volume were 0.8 ml/min and 20 µl, respectively. The chromatographic peaks of the analysts were confirmed by comparing their retention time and UV spectra with those of the reference standards. Quantification was carried out by the integration of the peak using external standard method. All chromatographic operations were carried out at ambient temperature.

3. 3. 2. Preparation of samples for HPLC

Buffer solution pH 2.5. 2.033 g of sodium perchlorate in 900 mL of purified water, adjust the pH of the solution with perchloric acid to (2.5±0.05) and adjust the volume of the solution with purified water to 1000.0 mL.

Moving phase. To 750 mL of a buffer solution of pH 2.5 add 250 ml of acetonitrile, mix and filter through a filter with a pore size of 0.45 µm (25:75, v/v).

Test solution. A portion of morpholinium thiazotate 60.0 mg of the substance of active substance are dissolved in 100 mL of solvent, the volume of the solution is adjusted to 100.0 mL with the same solvent and mixed.

Reference solution A. Place 1.0 mL of the test solution in a 100.0 mL volumetric flask, make up to volume with the solvent and mix.

3. 3. 3. Chromatographic conditions GC/FID method

Analyses were performed using a GC-2010 Plus Shimadzu with flame ionization detector and autosampler AOC-20i+s (Shimadzu Technologies, Kyoto, Japan). A robotic autosampler and a split/splitless injection port were used. Injection port temperature was kept at 250 °C until the end of the analysis. The separation of analytes was carried out on a with Rxi-5 ms (Restek Corporation, Bellefonte, PA, USA, capillary column (30 m long, 0.25 mm outer diameter and 0.25 µm liquid stationary phase thickness) with a liquid stationary phase) 5 % diphenyl and 95 % polydimethylsiloxane) with helium at a purity of 99.999 % as the carrier gas in a constant flow of 1.49 mL/min. The oven temperature was programmed at 75 °C for 5 min, then increased to 290 °C at 10 °C/min and increased to 320 °C at 20 °C/min and kept for 10 min. The total time was 41 min. Injection volume was 1.0 µl, injection mode was split (split ratio 10), carrier gas – helium.

3. 3. 4. Preparation of GC/FID method

A portion of morpholinium thiazotate 30.0 mg of the substance of active substance are dissolved in 100 mL of solvent, the volume of the solution is adjusted to 100.0 mL with the same solvent and mixed. The subsequent solution was transferred to 200 µL insert placed autosampler vials, and 1 µL aliquot was injected into GC-MS system for analysis. The comparison of chromatographic responses was used to evaluate the extraction efficiency.

3. 4. Economic evaluation of developed methods

We conducted an analysis of national legislation to calculate the cost of analytical research for the methods of analysis (HPLC, GC-MS, GC-FID) [5]. According to the accounting regulations' standards, the costs included are direct labor costs, other direct costs, variable overhead, and fixed overhead [6]. Other expenses include the intra-factory movement of materials, semi-finished products, tools from warehouses to workshops, and finished products to warehouses and a lack of work in progress, payment for downtime, and so forth. The depreciation of fixed assets is a part of the overhead costs. It should be noted that the list and composition of items of cost products (works, services) are established by the enterprise independently. Therefore, considering the provisions of national legislation on determining the cost of products (works, services) and the specificity of analytical research, we have formed the following cost items: basic raw materials, auxiliary materials, electricity, transport and procurement costs, wages, social security payments, fixed assets, recycling [7].

The calculations were made considering the time of analytical investigation for each method, namely HPLC analysis-10 min, GC-MS-45 min, FID-45 min, and the cost of the calculations are presented in Euro. The conversion was carried out according to the rate of the National Bank of Ukraine on 2021, 1 Euro=30.22 UAH [8].

3. 5. Assessment of Analytical Methods Impact on Environmental

The influence of the analytical methods on environmental depends on its analytical procedure parameters, such as the number of reagents, hazards, energy, and waste [9]. The comparison of methods was performed using Eco-scale, where the ideal green analysis has a value of 100. If some parameter of the method departs from the principles of the ideal green analysis, penalty points are assigned. The sum of the penalty points received after revision should be used for the Eco-scale calculation, according to the following formula:

$$\text{Analytical Eco-scale} = 100 - \text{penalty points}. \quad (1)$$

The results of an investigation can be ranked in the following matter:

1) >75 represents excellent green analysis;
2) >50 represents acceptable green analysis;
3) <50 represents unacceptable green analysis.

The Globally Harmonized System of Classification and Labelling of Chemicals provides full information about the determination of the safety class of the reagent based on physical, environmental, and health hazards [10]. For simplicity, some papers propose a calculation of penalty points for each reagent by multiplying the number of Globally Harmonized System (GHS) hazard pictograms by a degree of hazard (for the mark "warning" multiplication by 1 and for "danger" – 2).

Penalty points for energy are assigned according to energy-consuming laboratory practices and instruments [11]. Thus, the least energy consuming methods (<0.1kWh per sample) are immunoassays, titration, UV-Vis spectrophotometer, UPLC, HPTLC, and other techniques which require more energy usage (≤1.5 kWh per sample) such as LC or GC. The most energy-consuming (>1.5 kWh per sample) are NMR, GC-MS, LC-MS, X-ray diffraction.

Furthermore, penalty points are assigned for occupational hazards and the generation of wastes.

4. Results

Currently, there are various pharmacopoeia methods such as visible spectroscopy, UV, IR, 1H NMR, GMS, TLC, GC, HPLC, potentiometric methods for the determination of morpholinium thiazotate.

Green chemistry is relevant and the interest in it is growing, so there is a demand to develop more **environmentally friendly** and **cost-efficient** methods for the analysis of medicines. After the development of methods, it is logical to conduct a comparative and economic analysis of the developed methods to establish the most advantageous of the existing methods. The new methodology should be specific enough to give accurate results and provide good separation, minimize amount of solvent and analysis time.

Using the HPLC method with isocratic elution, the analysis time of morpholinium thiazotate was 3 minutes, the retention time – about 2.5 min (Fig. 2).

During the development of the HPLC assay, the optimal chromatographic conditions were selected: column type, composition of the mobile phase, elution conditions (Fig. 3).

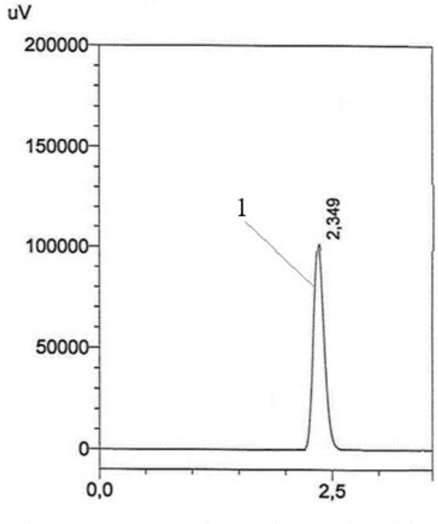

Fig. 2. Chromatograms of samples analysed by HPLC method morpholinium thiazotate at 205 nm

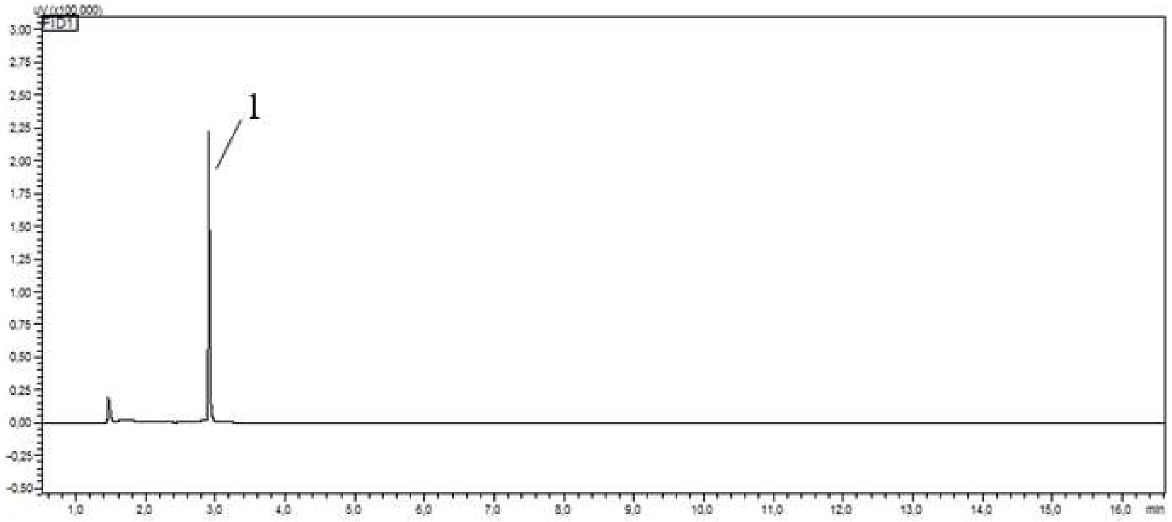

Fig. 3. Chromatograms of samples analysed by GC-FID method morpholinium thiazotate (1)

In addition, in the developed GC-FID method firstly analysis time and cost of analysis were reduced, so it was selective, sensitive and accurate in the same time. The GC-FID analysis method has a very high linear dynamic range (up to 107), which gives it many advantages in quantitative analysis.

This study shows that HPLC, GC-FID methods for the quantitative analysis of morpholinium thiazotate were developed and approved in accordance with the International Conference on Harmonization and the State Pharmacopoeia of Ukraine [12].

4. 1. Method validation

The developed methods were validated in terms of specificity, linearity, precision, stability, and accuracy, besides the Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated for both methods.

4. 1. 1. Specificity

The interference of placebo excipients for capsules and syrup was investigated by comparing the chromatograms obtained from test, standard, and placebo solutions. The prepared placebo contained the same ingredients in an equal amount as test samples. Obtained results showed no interference to morpholinium thiazotate from excipients. For this reason, the proposed methods are specific for the determination of morpholinium thiazotate.

4. 1. 2. Linearity, LOD, LOQ

The study of linearity was carried out by the analysis of a series of solvents with different concentrations of morpholinium thiazotate. The results of linearity studied with the calculated LOD and LOQ are shown in Table 1.

Table 1

Obtained results from studying linearity, LOD and LOQ

Method	Calibration curve	Correlation Coefficient r^2 (n=3)	Linear Range (μg/mL)	RSD (%)	LOD (μg/mL)	LOQ (μg/mL)
HPLC	y=0.9987x+3.428	0.9998	10–150	1.57	2.74	7.5
GC-FID	y=(5.51858e+007)x–24968.4	0.9987	0.12–16	2.2	0.046	0.115

4. 1. 3. Precision

The study has been carried out within two days by different analysts. Test solution with 100 % concentration was analysed. The results of the RSD deviation of assay determination, also the errors of the method, are shown in Table 2. The developed method is correct since the requirements for the error criterion are ≤6.4 %.

Table 2

Results of precision study for HPLC and GC-FID methods

Method	Concentration (μg/mL)	Intra-Day (n=6)			Inter-Day (n=12)		
		Found±S.D., %	RSD, %	Error, %	Found±S.D., %	RSD, %	Error, %
HPLC	600	100.8±0.45	0.54	0.45	100.35±0.25	0.79	0.98
GC-FID	16	100.12±0.24	0.52	0.45	100.25±0.34	0.63	0.63

4. 1. 4. Accuracy

The investigation of accuracy has been evaluated by means of recovery assays carried out by adding known amounts of the reference compound to the sample solutions. The amounts of analysts added correspond to 50 %, 100 %, and 200 % of morpholinium tiazotate in samples. The calculated data are shown in Table 3. Recoveries were obtained in the range of 98.13–100.12, depicting that the proposed methods are accurate for the determination of morpholinium thiazotate.

The stability of morpholinium thiazotate was carried out within 24 h for a standard solution. It was established that stored solutions were stable for up to 24 h in the case of the HPLC, GC-FID methods, hence peak deviations of substance were 0.395 %, 0.387 %, respectively.

All procedures showed satisfactory results in the data and could be recommended for the analysis of morpholinium thiazotate in different dosage forms as accurate and reproducible methods of quality control.

Table 3

Accuracy of the developed method

Reference Value (%)	Amount Measured (%)		Relative Standard Deviation (%)		Recovery (%)	
	HPLC	GC-FID	HPLC	GC-FID	HPLC	GC-FID
50 %	50.08	49.91	0.13	0.06	100.16	99.82
100 %	100.21	99.80	0.07	0.11	100.21	99.80
200 %	196.70	199.83	0.21	0.15	98.35	99.92

4. 2. Eco-Scale Calculation

Chromatographic analysis requires the usage of various procedures and pre-treatment of analysed samples. Besides, for the assay of a sample, usually, a couple of determination methods are acceptable. The selection of analytical methods is commonly based on its accuracy, precision, cost, and the environmental and health impact [13].

All developed chromatographic methods for the analysis of morpholinium thiazotate were estimated for their greenness using the analytical Eco-scale to choose the method with the least environmental impact. Furthermore, different conditions of pre-treatment for each sample were considered. HPLC (Table 4) and GC-MS with FID (Table 5) showed similar Eco-scale values.

Thus, the HPLC for morpholinium thiazotate has 16, respectively, compared to the GC-FID detector has 9, respectively. Both methods provide excellent green analysis, but the differences are significant, so GC-FID detector will have advantages in terms of green chemistry. Thus, the GC-FID method was recognized as the less greenish technique compared to HPLC.

Table 4

Calculation of penalty points for the HPLC method in the analysis of one sample

Reagents	Penalty points
Acetonitrile 2.0 mL	4
Water 8 mL	0
Instruments	
HPLC	1
Waste	8
Occupational hazard	3
Sample preparation	
Water 50 mL	0
Total penalty points:	16
Analytical Eco-Scale total score:	78

Table 5

Assessment of GC-FID detector analysis of one sample

Reagents	Penalty points
Water 8 mL	0
Instruments	
GC-MS	3
Waste	4
Occupational hazard	0
Sampler	1
Transport	1
Sample preparation	
Water 50 mL	0
Total penalty points:	9
Analytical Eco-Scale total score:	35

4. 3. Economic Calculation of Analytical Methods

The cost of required reagents for analytical research was carried out based on the official company database of Sigma-Aldrich [14]. The procedure of the cost calculation is presented in Table 6.

Table 6

Cost expenses under “basic raw materials and materials” and “supporting materials”.
Calculation unit – 1 sample analysis

Raw Material	Price, Euro	Quantity for Conducting 1 Test Sample	Cost, Euro
HPLC Method			
The Main Raw Materials			
Acetonitrile 2.5 L	€332.00	2 mL	0.66
ZORBAX SB-C18, 80Å, 5 µm, 4,6×150 mm	€610.00	1	6.1
Water for chromatography 1 L	€31.90	8 mL	0.51
Membrane filter 0,45 microns No. 100	€250.00	1	0.25
Sodium perchlorate monohydrate 250 gr	€63.90	2 g	1.02
Vials, volume 2 mL, No. 100	€23.50	1	2.35
Total			10.89
Supporting Materials			
Latex gloves with powder No. 100	€12.56	1	0.13
Disposable non-woven medical cap No. 100	€2.50	1	0.03
Shoe covers medical sterile No. 50	€3.5	1	0.07
Total			0.23
GC-FID			
The Main Raw Materials			
Membrane filter 0,45 microns No. 100	€223.00	0.1	2.23
Helis, 9100 L	€480.00	0.615 L	0.032
Vials, volume 2 mL, No. 100	€23.50	1	2.35
Total			4.61
Supporting Materials			
Latex gloves with powder No. 100	€12.56	1	0.13
Disposable non-woven medical cap No. 100	€2.50	1	0.03
Shoe covers medical sterile No. 50	€3.5	1	0.07
Total			0.23

Costs for “electricity” are the costs that are directly spent in the technological process when performing analytical research. Electricity costs for technological purposes are calculated according to the actual cost of energy based on the readings of the measuring instruments. Therefore, the electricity cost was calculated based on the technical characteristics of the equipment (energy consumption per kWh), the period of the analytical study, and the national electricity tariff for household and small non-household consumers at 0.12 Euro/kWh with a value-added tax. It is established that the number of costs for the article "electricity" in the conduct of analytical research by HPLC is 0.05 Euro, GC-FID is 0.21 Euro.

Transport and procurement costs according to various data can be from 1 % to 15 % of the total cost of raw materials, basic and auxiliary materials. To calculate the cost of analytical research for methods of analysis HPLC, GC-FID we have taken the minimum values of transport and procurement costs, namely 1 % [14].

The calculation of expenditures under the article "wages" was made based on official data of the State Statistics Service as of 01.12.2021 with respect to the average wage per month of the Senior Researcher, which was 281 euros. For a month of work, the senior researcher works 176 h. Allowances for social events make up 38.5 % of the salaries of the main employees.

For the calculation of the article "Depreciation of fixed assets," we used a straight-line method of calculating depreciation. Under this method, the annual depreciation amount (ADA) is determined by dividing the amortized cost (AC) by the useful life (UL). According to accounting standards 7, the amortized cost of an item of equipment is the initial or revalued value (RV) of assets less the liquidation value (LV), with the following calculation formula:

$$AC=RV-LV. \tag{2}$$

According to the provisions of item 138.3.3 of the Tax Code of Ukraine, the minimum allowable depreciation terms for fixed assets for equipment are five years. Therefore, considering the data of the initial and liquidation value of the equipment, which is necessary for the analytical study and the minimum useful life, we have calculated the depreciation of fixed assets [15]. It is established that the amount of expenses under the item "depreciation of fixed assets" in the conduct of analytical research by HPLC is 1.05 Euro, GC-FID is 0.17 Euro.

The environmental impact of chemical reagents is a global environmental and pharmaceutical problem worldwide. Protecting the environment from the adverse effects of drugs requires immediate resolution. The laboratory enters into an agreement with an organization licensed to utilize reagent waste. According to the commercial offers of Kharkiv-Eco LLC, the cost of services related to the organic solvent waste is EUR 0.55 per 1 L. It was found that the number of disposal costs HPLC exploration was 0.01 Euro, GC-FID method was 0.0003 Euro (Table 7).

The "basic raw materials" are the largest share in the structure of total expenses. Thus, for the HPLC study, their specific gravity is 91.10 %, the GC-FID is 57.70 %. It should be noted that the article "depreciation of fixed assets" is a significant expense in the cost of analytical research in various methods. It can be explained by the high cost of the equipment. Based on the results, the GC-FID method had the lowest cost. The costs for applying the GC-FID method was 2.3 and 1.6 times lower than for HPLC, respectively.

Table 7

The results of the calculation of cost items by various methods of analytical research are summarized

No.	Cost Articles	HPLC Method (10 min)		GC-MS Method (41 min) with FID	
		Price, Euro,	Share, %	Amount, Euro	Share, %
1	The main raw materials	10.89	91.1	4.61	57.70
2	Supporting materials	0.23	1.93	0.23	2.88
3	Electricity	0.05	0.42	0.21	2.63
4	Transportation and procurement costs	0.04	0.34	0.02	0.25
5	Deductions for social events	0.21	1.76	0.11	1.38
6	Salary	0.27	2.266	1.09	13.64
7	Depreciation of fixed assets	0.25	2.09	1.72	21.53
8	Recycling of waste chemicals	0.01	0.08	0.0003	0.004
9	Total cost	11.95	100	7.99	100

4. 4. Green Assessment

Despite several reported approaches for determining greener nature of analytical procedures [9, 16–18], only the "AGREE methodology" [9] employs all 12 GAC principles. Accordingly, the greenness nature of the present approach was assessed utilizing "AGREE Calculator". Fig. 4 depicts the overall AGREE scale for the present analytical approach. Fig. 4 lists the AGREE report sheet and AGREE score for each GAC principle.

The overall AGREE scale for the proposed analytical methods HPLC, GC-FID was calculated as 0.62, 0.72, respectively, indicating that the proposed analytical methods for the analysis of morpholinium thiazotate are extremely green, but the method is GC-MS with detector FID still greener.

a *b*

Fig. 4. Analytical GREEnness (AGREE) scale for the greener: *a* – normal-phase HPLC, *b* – GC-FID approach

5. Discussion

The obtained results demonstrate that the HPLC method is more linear (R^2=0.9998>0.994), repeatable (RSD=1.57<1.6 %) and specificity is comparable to the HPLC method, as described in the literature [19–21].

However, the method may have shortcomings, which are characteristic of the HPLC method, for example, it is necessary to overcome the cost of ownership and take a lot of time, so we use the splitting method GC-FID, which may be possible to control morpholinium tiazotate, and may cost less from the HPLC method.

After conducting economic calculations, it became clear the GC-FID method had the lowest expenses. The costs for applying the GC-FID method were 2.3 times lower than for HPLC.

The GC-FID method is more ecological as its estimation on a analytical GREEnness (AGREE) scale has made 0.73 it more than in HPLC 0.62.

Study limitations. The proposed methods for the quantification of morpholinium thiazotate have not been studied to determine API data in the presence of other drugs and in environmental samples.

The prospects for further research lie in the development of various methods for the analysis of samples taken from the most important medium (soil, waste water).

6. Conclusions

A reliable HPLC, GC-FID method for routine qualitative and quantitative analysis of morpholinium thiazotate was developed and validated in terms of specificity, linearity, precision, accuracy, and stability.

Furthermore, the LOD and LOQ were calculated for methods. These methods offer a useful analytical tool for routine quality control of morpholinium thiazotate in different samples. Additionally, the environmental impact of each method was estimated and the HPLC method was recognized as a less greenish method than GC-MS and GC-MS with FID.

The GC-FID method had the lowest expenses. The costs for applying the GC-FID method were 2.3 times lower than for HPLC. According to the obtained results, the GC-FID method was recognized as the most suitable for analysis, since it has a lower influence on the environment than the HPLC method, needs smaller costs for applying than GC-FID, and still shows satisfactory accuracy. The constructed experiment is intended for further application of the technique in solving environmental issues, namely environmental pollution by drugs.

Conflict of interests

The authors declare that they have no conflicts of interest.

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