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ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY METHODS FOR THE DETERMINATION OF THE RESIDUAL QUANTITIES OF RAMIPRIL AND HYDROCHLOROTHIAZIDE FOR CONTROLLING THE CLEANING OF EQUIPMENT

Kateryna Typlynska, Yuliya Kondratova, Mariana Horyn, Liliya Logoyda

Monitoring the completeness of equipment cleaning is essential to prevent cross-contamination of medicinal products. Therefore, it is necessary to develop fast and sensitive methods for studying residual quantities of active ingredients on the surfaces of technological equipment.

The aim of the work *was to develop and validate analytical methods for the determination of ramipril and hydrochlorothiazide in wash waters by ultra-performance liquid chromatography–mass spectrometry method.*

Materials and methods. *In the study, standard samples of ramipril (USP RS) and hydrochlorothiazide (USP RS), as well as class A reagents, were used. Samples were analysed on a liquid chromatograph with an MS detector (Agilent 6420 and Waters Xevo TQD ACQUITY). We used the Kinetex C18 column (2.1 mm×30 mm×1.7 μm); mobile phase – 0.1 % formic acid solution in deionised water – Acetonitrile (ratio 73:27 for the determination of ramipril and 91.5:8.5 for the determination of hydrochlorothiazide); mobile phase rate of 0.4 mL/min for the determination of ramipril and 0.35 mL/min for the determination of hydrochlorothiazide; column temperature 45 °C for the determination of ramipril and 40 °C for the determination of hydrochlorothiazide, ionisation mode – electric spray in positive mode; The detection parameters are the mode of registration of the daughter ion 417→234 m/z for the determination of ramipril and 298→281 m/z for the determination of hydrochlorothiazide.*

Results and discussion. *Methods for the determination of ramipril and hydrochlorothiazide in wash waters by ultra-performance liquid chromatography–mass spectrometry have been developed. The developed methods have sufficient linearity, correctness and precision. The sensitivity of the techniques was confirmed at the level of 0.0026 μg/ml. The techniques can be used in the concentration range of 0.0026–0.0255 μg/ml*

Conclusions. *Analytical methods for determining ramipril and hydrochlorothiazide in wash waters have been developed and validated.*

Keywords: *hydrochlorothiazide, ramipril, equipment cleaning control, validation, UPLC*

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1. Introduction

Equipment cleaning and control of the completeness of equipment cleaning is an important step in preventing contamination of the medicinal product with a previously manufactured medicinal product. Each manufacturer must ensure that after cleaning, no active ingredient or detergent residues beyond the permissible maximum remain on the surface of the equipment [1]. The completeness of cleaning of the equipment is monitored by analysing the most recent washing water or by analysing the washes from the surface of the equipment. The methods used to analyse wash water should be highly selective and sensitive. For the development of such techniques, methods of high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC) with spectrophotometric or mass spectrometric (MS) detection, gas chromatography, ion exchange chromatography, atomic absorption spectroscopy and UV spectroscopy are used. Due to insufficient sensitivity, UV spectroscopy cannot be used for highly active substances. Ion exchange chromatography and atomic absorption spectroscopy are usually used to control the residual content of detergents [2] or preparations containing metal ions [3]. Gas chromatography can only be used to monitor volatile compounds. The methods of HPLC and UPLC with spectrophotometric detection are the most widespread; however, their use is limited for highly active compounds and compounds with weak chromatophores [4]. Ramipril (2S,3aS,6aS)-1-[(2S)-2-[[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-3,3a,4,5,6,6a-hexahydro-2H-cyclopenta[b]pyrrole-2-carboxylic acid is a prodrug and non-sulfhydryl ACE inhibitor with antihypertensive effect. It is metabolised to ramiprilat in the liver and kidneys. Hydrochlorothiazide, 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide, is a diuretic that is often used to treat hypertension and oedema caused by fluid retention. Ramipril and hydrochlorothiazide can be used as mono preparations or in combination. The combination of these drugs has been well studied and has not lost its relevance for more than

30 years [5]. In the scientific literature, analytical methods for the determination of ramipril and hydrochlorothiazide in medicinal products are presented; however, no analytical method for controlling the cleaning of equipment is described [5–27].

The **aim of this study** was to develop and validate an analytical methods for the determination of ramipril and hydrochlorothiazide in washing waters by the method of UPLC-MS.

2. Planning of the research

The methodology of the research includes:

1. Analysis of the scientific literature.

2. Selection of optimal chromatographic conditions (choice of detector, column, composition of mobile phase, column temperature, flow rate).

3. Performance of the analytical methods on Agilent 6420 MS- detector with validation study.

4. Performance of the analytical methods on Waters Xevo TQD ACQUITY MS- detector with validation study.

3. Materials and methods

The research was conducted in the period 2019–2022.

Apparatus.

Liquid chromatograph with MS-detector Agilent 6420 (USA), liquid chromatograph with MS- detector Waters Xevo TQD ACQUITY (USA). Analytical balance Mettler Toledo XPE-205 (Switzerland) and Sartorius AG CP224S (Germany). A Kinetex C18 column (2.1 mm×30 mm×1.7 μm) was used for both substances. Chromatographic conditions for the determination of ramipril: mobile phase 0.1 % solution of formic acid in deionised water – acetonitrile (73:27); mobile phase speed – 0.4 ml/min; column temperature – 45 °C; ionisation mode – electrospray in positive mode; detection parameters – daughter ion registration mode 417→234 m/z. Chromatographic conditions for the determination of hydrochlorothiazide: mobile phase 0.1 % solution of formic acid in deionised water – acetonitrile (91.5:8.5); mobile phase speed – 0.35 ml/min; column temperature – 40 °C; ionisation mode – electrospray in positive mode; detection parameters – daughter ion registration mode 298→281 m/z.

Chemicals and materials.

Ramipril (purity≥99 %, HPLC) and hydrochlorothiazide (purity≥99 %, HPLC) were purchased from AARTI Industries Limited (India). The used chemicals: acetonitrile (Honeywell), formic acid (Honeywell), phosphoric acid (Sigma-Aldrich), ethyl alcohol (Ukrspirt). The demineralised water used for analyses was in-house product of Stilman with conductivity of less than 0.5 μS/cm.

Preparation of solutions.

Ramipril reference solution: 5.0 mg of ramipril was dissolved in 30 ml of ethyl alcohol and the volume of the solution was adjusted to 50.0 ml with water. Using the original solution, a solution was prepared containing 0.01 μg/ml of ramipril in a mixture of acetonitrile: water (5:95).

Hydrochlorothiazide reference solution: 5.0 mg of hydrochlorothiazide was dissolved in 40 ml of ethyl alcohol, and the volume of the solution was adjusted to 50.0 ml with water. Using the original solution, a solution containing 0.01 μg/ml of hydrochlorothiazide in 0.05 % phosphoric acid was prepared.

Test solution for the determination of ramipril: 1.1 ml of analysed washing water was adjusted to 20.0 ml with a mixture of acetonitrile: water (5:95).

Test solution for the determination of hydrochlorothiazide: 0.85 ml of analysed washing water was adjusted to 50.0 ml with 0.05 % phosphoric acid.

For validation using Agilent 6420, 10 model solutions were prepared for both substances in the concentration range of 0.0026–0.0255 μg/ml (equivalent to 25–247 % of the lower limit of the range of acceptance criteria for ramipril and 25–253 % of the lower limit of the range acceptance criteria for hydrochlorothiazide).

To confirm the reproducibility of the technique using Waters Xevo TQD ACQUITY, 5 model solutions were prepared in the concentration range of ramipril 0.0026–0.0250 μg/ml (equivalent to 25–242 % of the lower limit of the range of acceptance criteria) and hydrochlorothiazide 0.0026–0.0255 μg/ml (equivalent to 25–250 % of the lower limit of the range of acceptance criteria).

4. Results

4. 1. Method performed on Agilent 6420 MS-detector

The development and validation of the method were carried out using a liquid chromatograph with an Agilent 6420 MS- detector. The content of acetonitrile in the mobile phase was varied in such a way that the retention time of hydrochlorothiazide was from 0.5 to 1.5 min. The mass of 417 m/z for ramipril corresponds to the [M+] molecular ion. The daughter ion (234 m/z) is formed by cleavage from the parent CO ion and part of the molecule with the gross formula $C_8H_{13}NO_2$ [8]. The mass of 298 m/z for hydrochlorothiazide corresponds to the molecular ion [M+]. The daughter ion (281 m/z) was formed by cleavage of the amino group. The lowest permissible content of ramipril in the washing solution was 0.188 μg/ml, hydrochlorothiazide – 0.592 μg/ml. The dilution of the washing solutions was chosen in such a way that the nominal concentration of the analyte in the tested solution was 0.010 μg/ml. To investigate the specificity of the method for determining ramipril, the solvent (mixture acetonitrile:water (5:95)) and the comparison solution were analysed. Typical chromatograms are shown in Fig. 1. The solvent (0.05 % phosphoric acid) and the reference solution were analysed to study the specificity of the hydrochlorothiazide determination method. Typical chromatograms are shown in Fig. 2.

The chromatogram of the solvent did not reveal peaks that could interfere with the peak of ramipril or hydrochlorothiazide. Linearity, accuracy and precision were investigated by a combined experiment in the concentration range of 0.0026 - 0.0255 μg/ml (for both ramipril and hydrochlorothiazide). Linear regression parameters were calculated. According to the obtained regression equation, the "found" value of the peak areas was calculated according to the formula:

$$s_i = a + b \cdot C_i,$$

where s – the value of the peak areas, calculated according to the regression equation;

C_i – analyte concentration in the corresponding solution for linearity research, μg/ml;

a – slope of the regression line;

b – y-intercept.

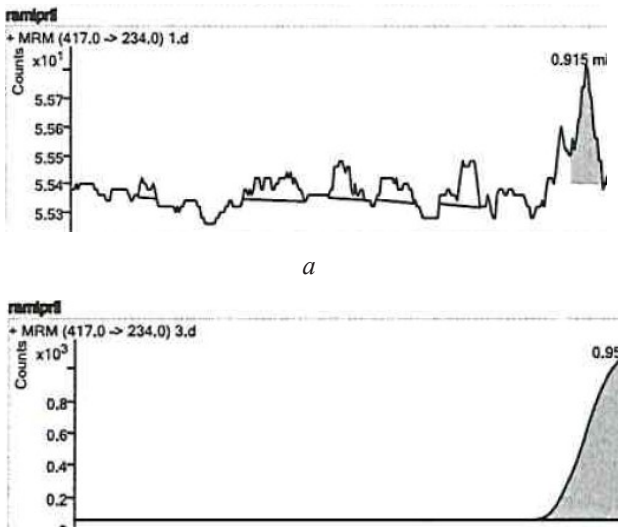


Fig. 1. Examples of chromatograms of ramipril obtained using Agilent 6420: *a* – chromatogram of solvent; *b* – chromatogram of the comparison solution

The calculation of the normalised value of the slope of the regression line (as a percentage of the signal of the equipment corresponding to the nominal concentration of the analyte in the solution) was carried out according to the formula:

$$|a_i|, \% = \left| \frac{a \cdot C_0}{S_0 \cdot C_{nom}} \right| \cdot 100 \%,$$

where S_0 – the area of the analyte peak in the comparison solution;

C_0 – analyte concentration in the comparison solution (0.01 μg/ml);

C_{nom} – the analyte concentration equivalent to the smallest content criterion in the washing solution (0.01 μg/ml).

The found analyte concentration in model solutions was calculated using the formula:

$$C_{m\_i} = \frac{C_0 \cdot S_i}{S_0},$$

where S_i – the analyte area in the corresponding model solution.

The ratio "Found"/"Put" was calculated according to the formula:

$$\text{Recovery}_i = \frac{C_{m\_i}}{C_{t\_i}} \cdot 100 \%,$$

where C_t – the given concentration in model solutions.

Chromatographic results and calculations for ramipril are given in Table 1, for hydrochlorothiazide – in Table 2. The graph of the dependence of the response of the signal on the concentration of ramipril in model solutions is shown in Fig. 3, hydrochlorothiazide – in Fig. 4. For ramipril, the correlation coefficient of the calibration line was 0.99986, which satisfies the acceptance criterion (not less than 0.995). The ratio of the slope of the regression line to the nominal concentration of ramipril was 4.041 %, which satisfies the acceptance criterion (no more than 5.0 %). The residuals are randomly scattered around zero. That is, the method was linear in the studied range.

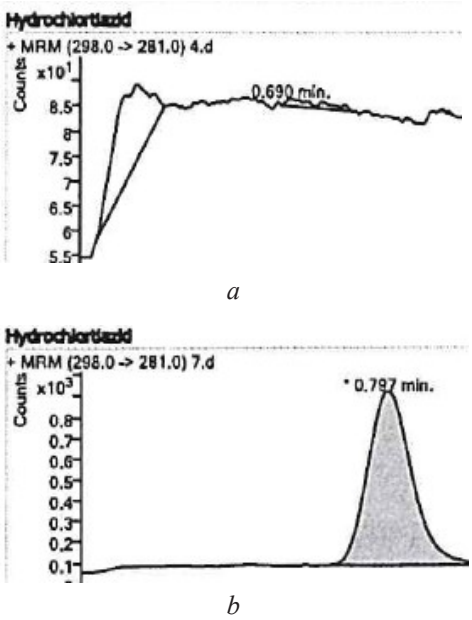


Fig. 2. Examples of chromatograms of hydrochlorothiazide obtained using Agilent 6420: *a* – chromatogram of solvent; *b* – chromatogram of the comparison solution

Table 1

Study results for ramipril

Solution	C_t, μg/ml	S_i	RSD_S	s_i	S_i–s_i	C_m, μg/ml	Recovery
M1	0.0026	2569	0.586	2311	258	0.0026	102.416
M2	0.0051	5097	0.606	5033	64	0.0052	101.605
M3	0.0077	7652	0.356	7755	–103	0.0078	101.693
M4	0.0102	10278	0.500	10477	–199	0.0104	102.449
M5	0.0128	13042	0.342	13199	–156	0.0133	104.002
M6	0.0153	15885	0.405	15921	–36	0.0162	105.556
M7	0.0179	18616	0.800	18643	–27	0.0189	106.034
M8	0.0204	21375	0.316	21364	10	0.0217	106.529
M9	0.0230	24156	0.562	24086	70	0.0246	107.014
M10	0.0255	26928	0.529	26808	119	0.0274	107.364

For hydrochlorothiazide, the correlation coefficient of the calibration line was 0.99961, which satisfies the acceptance criterion (not less than 0.995). The ratio of the slope of the regression line to the nominal concentration of hydrochlorothiazide was 1.198 %, which satisfies the acceptance criterion (no more than 5.0 %). The residuals are randomly scattered around zero. That is, the method is linear in the studied range. Individual values of ramipril recovery

were in the range of 101.6–107.4 %, which satisfies the criterion of acceptability (90.0–110.0 %). The average value of the recovery rate was 104.5 %, which satisfies the acceptance criterion (95.0–105.0 %). Individual values of hydrochlorothiazide recovery were in the range of 97.6–102.3 %, which satisfies the criterion of acceptability (90.0–110.0 %). The average value of the recovery rate was 100.0 %, which satisfies the acceptance criterion (95.0–105.0 %). The accuracy of the method was sufficient. The relative standard deviation of the recovery of ramipril was 2.191 %, which satisfies the acceptance criterion (not more than 5.0 %). The relative standard deviation of the recovery of hydrochlorothiazide was 1.505 %, which satisfies the acceptance criterion (not more than 5.0 %). That is, the precision of the method was sufficient. The limit of detection (DL) and the limit of quantification (QL) were calculated based on the parameters of the calibration line in the concentration range (0.0026–0.0128 μg/ml) according to the formula:

$$QL = \frac{10 \cdot S_a}{b},$$

$$DL = \frac{3.3 \cdot S_a}{b},$$

where S_a – the deviation of the slope of the regression equation.

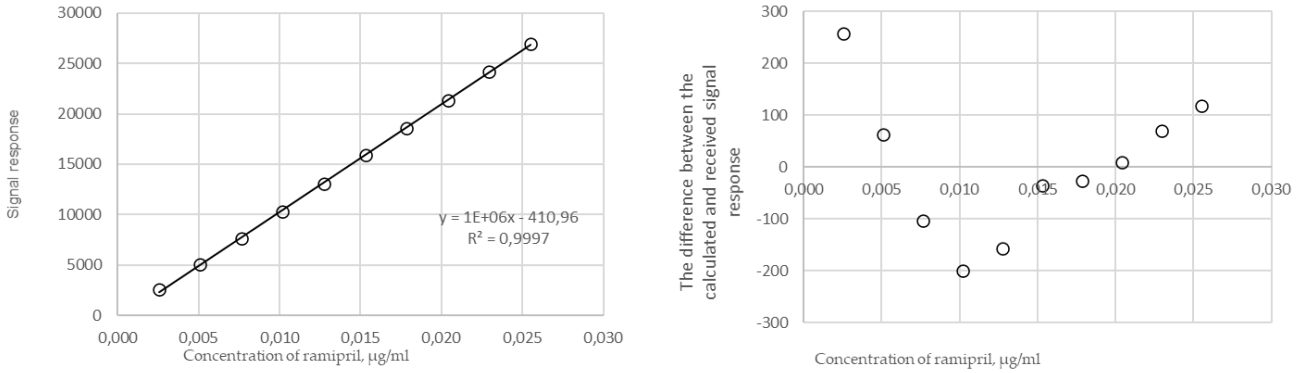
The results of the calculations are given in Table 3.

To confirm the *QL*, the signal-to-noise ratio, the degree of recovery, and the RSD between the degrees of recovery were calculated for model solution M1. Chromatographic results and calculations for ramipril are given in Table 4, for hydrochlorothiazide – in Table 5. *QL* of ramipril was confirmed at the concentration level of 0.0026 μg/ml, which was 25 % of the smallest criterion for the content of ramipril in the washing solution. The calculated value was almost three times less than the confirmed concentration; that is, at the lower limit of the studied range, the method was reproduced with a sufficient level of reliability. QL of hydrochlorothiazide was confirmed at the concentration level of 0.0026 μg/ml, which was 25 % of the smallest criterion for the content of hydrochlorothiazide in the washing solution. The calculated value was half the confirmed concentration; that is, at the lower limit of the studied range, the method was reproduced with a sufficient level of reliability.

Table 2

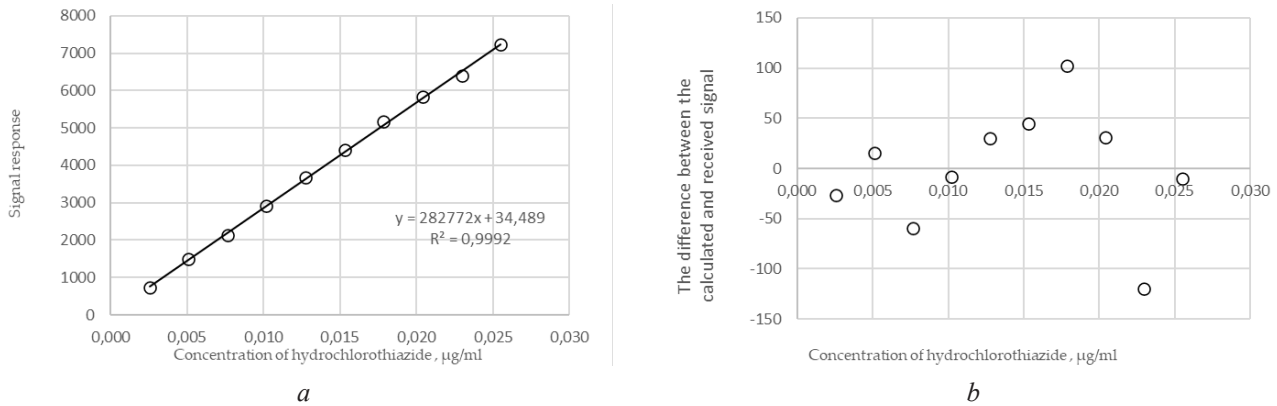
Study results for hydrochlorothiazide

Solution	C_i, μg/ml	S_i	RSD_S	s_i	S_i–s_i	C_m, μg/ml	Recovery
M1	0.0026	729	2.777	756	–26	0.0026	100.011
M2	0.0051	1492	1.106	1477	16	0.0052	102.320
M3	0.0077	2138	2.082	2198	–60	0.0075	97.726
M4	0.0102	2910	1.369	2919	–8	0.0102	99.771
M5	0.0128	3670	0.904	3640	30	0.0128	100.651
M6	0.0153	4405	0.386	4361	44	0.0154	100.682
M7	0.0179	5184	0.947	5082	102	0.0181	101.559
M8	0.0204	5834	0.338	5803	31	0.0204	100.006
M9	0.0230	6405	0.351	6524	–119	0.0224	97.584
M10	0.0255	7235	0.194	7245	–10	0.0253	99.212



a *b*

Fig. 3. Graph of dependence of signal response on ramipril concentration: *a* – regression graph; *b* – residual graph



a *b*

Fig. 4. Graph of dependence of signal response on hydrochlorothiazide concentration: *a* – regression graph; *b* – residual graph

Table 3

Calculation results of DL and QL

Analyte	b	S_a	DL, μg/ml	QL, μg/ml
Ramipril	1024654	91	0.0003	0.0009
Hydrochlorothiazide	286248	39	0.0005	0.0014

Table 4

Confirmation of QL of ramipril

Parameter	Solution	M1		
		1	2	3
Concentration, μg/ml		0.0026		
Peak area		2586	2559	2561
Individual values of the recovery	Criterion	90.0–110.0 %		
	Results	103.107	102.030	102.110
RSD between degrees of recovery	Criterion	≤10.0 %		
	Results	0.586 %		
Signal-to-noise ratio	Criterion	≥10		
	Results	642.69	804.84	619.24

Table 5

Confirmation of QL of hydrochlorothiazide

Parameter	Solution	M1		
		1	2	3
Concentration, μg/ml		0.0026		
Peak area		713	723	752
Individual values of the recovery	Criterion	90.0–110.0 %		
	Results	97.772	99.143	103.120
RSD between degrees of recovery	Criterion	≤10.0 %		
	Results	2.8 %		
Signal-to-noise ratio	Criterion	≥10		
	Results	41.44	53.81	66.86

4. 2. The method performed on Waters Xevo TQD ACQUITY MS- detector

We investigated the possibility of reproducing the methods on another equipment (a liquid chromatograph with a Waters Xevo TQD ACQUITY MS-detector). Typical chromatograms of the solvent and reference solution for ramipril are shown in Fig. 5, for hydrochlorothiazide – in Fig. 6.

The chromatogram of the solvent did not reveal peaks that could interfere with the peak of ramipril or hydrochlorothiazide. Linearity, accuracy and precision were investigated by a combined experiment in the concentration range of ramipril 0.0026–0.0250 μg/ml and hydrochlorothiazide 0.0026–0.0252 μg/ml. Chromatographic results and calculations for ramipril are shown in Table 6, for hydrochlorothiazide – in Table 7.

Table 6

Study results for ramipril

Solution	C_t, μg/ml	S_i	RSD_S	s_i	S_i–s_i	C_m, μg/ml	Recovery
M1	0.0026	705	1.349	701	4	0.0025	97.115
M2	0.0054	1500	1.470	1494	6	0.0054	99.250
M3	0.0109	3023	1.288	3020	3	0.0109	100.000
M4	0.0217	5995	0.941	6073	–78	0.0215	99.140
M5	0.0250	7053	2.311	6988	65	0.0253	101.433

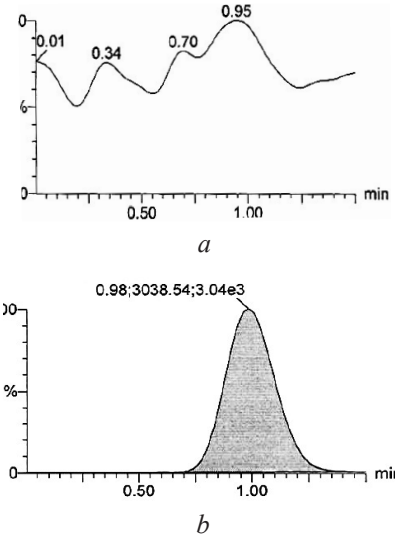


Fig. 5. Examples of chromatograms of ramipril obtained using Waters Xevo TQD ACQUITY: *a* – chromatogram of solvent; *b* – chromatogram of the comparison solution

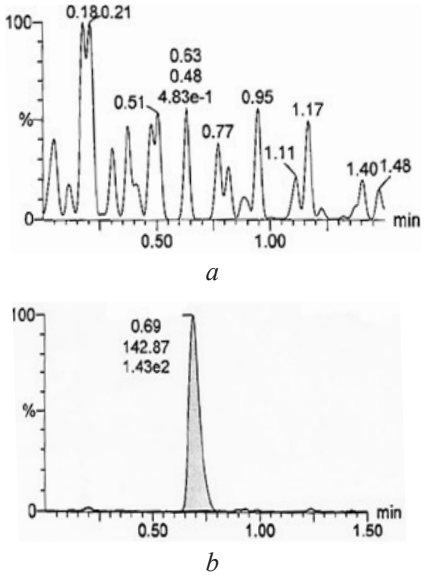


Fig. 6. Examples of chromatograms of hydrochlorothiazide obtained using Waters Xevo TQD ACQUITY: *a* – chromatogram of solvent; *b* – chromatogram of the comparison solution

Table 7

Study results for hydrochlorothiazide

Solution	C_t, μg/ml	S_i	RSD_S	s_i	S_i–s_i	C_m, μg/ml	Recovery
M1	0.0026	33	3.464	33	1	0.0026	98.270
M2	0.0055	69	1.449	70	–1	0.0053	97.642
M3	0.0109	141	1.473	142	–1	0.0109	100.000
M4	0.0219	293	1.423	287	6	0.0227	103.538
M5	0.0252	325	2.263	330	–5	0.0252	100.003

The graph of the dependence of the response of the signal on the concentration of ramipril in model solutions is shown in Fig. 7, hydrochlorothiazide – in Fig. 8. For ramipril, the correlation coefficient of the calibration line was 0.99983, which satisfies the acceptance criterion (at least 0.995). The ratio of the slope of the regression line

to the nominal concentration of hydrochlorothiazide was 1.121 %, which satisfies the acceptance criterion (no more than 5.0 %). For hydrochlorothiazide, the correlation coefficient of the calibration line was 0.99954, which satisfies the acceptance criterion (not less than 0.995). The ratio of the slope of the regression equation to the nominal concentration of hydrochlorothiazide was 1.567 %, which satisfies the acceptance criterion (no more than 5.0 %). The residuals were randomly scattered around zero. That is, the method was linear in the studied range.

Individual values of ramipril recovery were in the range of 97.1–101.4 %, which satisfies the criterion of acceptability (90.0–110.0 %). The average value of the degree of recovery was 99.4 %, which satisfies the acceptance criterion (95.0–105.0 %). Individual values of hydrochlorothiazide recovery were in the range of 97.6–103.5 %, which satisfies the acceptance criterion (90.0–110.0 %). The average value of the degree of recovery was 99.9 %, which satisfies the acceptance criterion (95.0–105.0 %). That is, the accuracy of the method was sufficient. The relative standard deviation of the recovery rates of ramipril was 1.275 %, which satisfies the acceptance criterion (not more than 5.0 %). The relative standard deviation of the recovery rates of hydrochlorothiazide was 2.294 %, which satisfies the acceptance criterion (not more than 5.0 %). That is, the precision of the method is sufficient. To confirm QL, the signal-to-noise ratio, the degree of recovery, and the RSD between the degrees of recovery were calculated for model solution M1. Chromatographic results and calculations for ramipril are given in Table 8, for hydrochlorothiazide – in Table 9. QL at the concentration level of 0.0026 μg/ml was confirmed.

Table 8

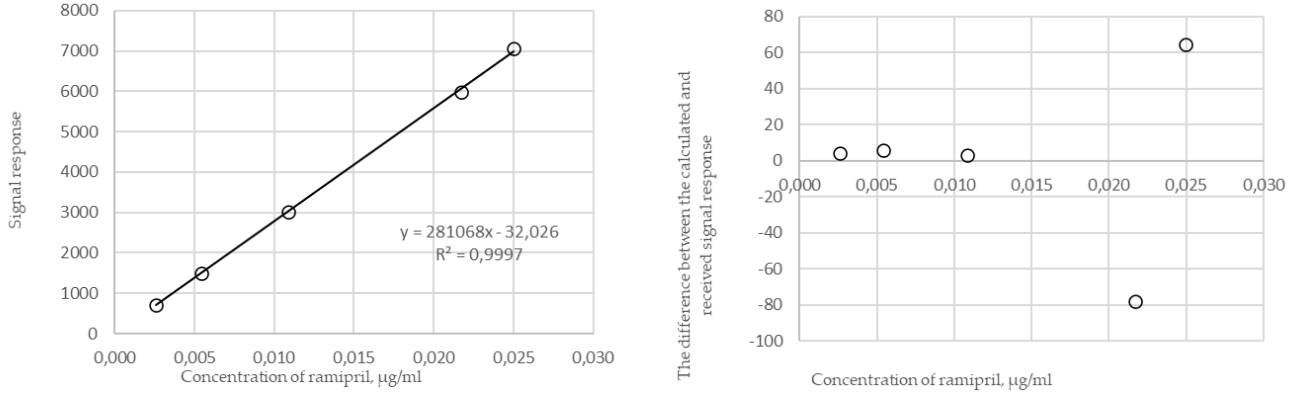
Confirmation of QL of ramipril

Parameter	Solution	M1		
		1	2	3
Concentration, μg/ml		0.0026		
Peak area		695	714	705
Individual values of the recovery	Criterion	90.0–110.0 %		
	Results	95.783	98.401	97.161
RSD between degrees of recovery	Criterion	≤10.0 %		
	Results	1.349		
Signal-to-noise ratio	Criterion	≥10		
	Results	668	681	602

Table 9

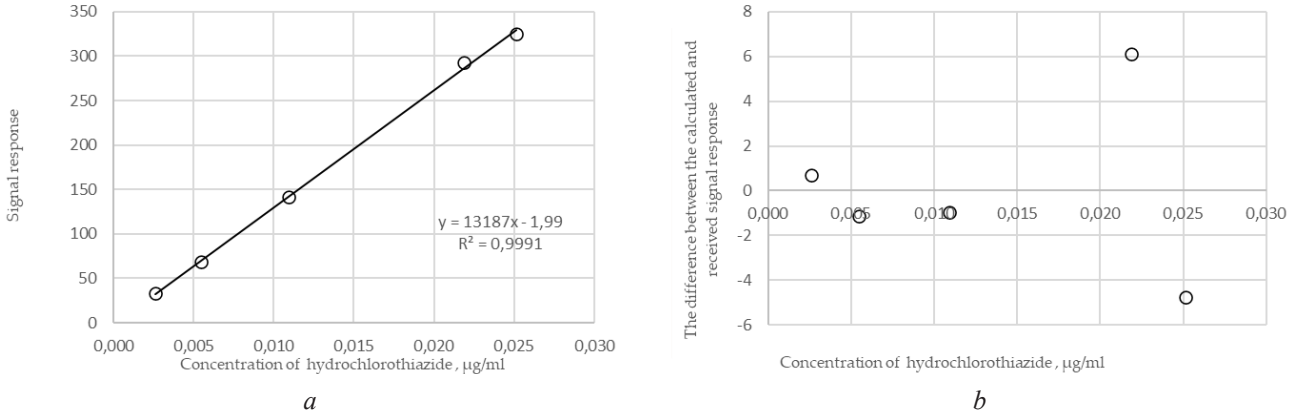
Confirmation of QL of hydrochlorothiazide

Parameter	Solution	M1		
		1	2	3
Concentration, μg/ml		0.0026		
Peak area		34	34	32
Individual values of the recovery	Criterion	90.0–110.0 %		
	Results	100.236	100.236	94.340
RSD between degrees of recovery	Criterion	≤10.0 %		
	Results	3.464		
Signal-to-noise ratio	Criterion	≥10		
	Results	127	140	135



a *b*

Fig. 7. Graph of dependence of signal response on ramipril concentration: *a* – regression graph; *b* – residual graph



a *b*

Fig. 8. Graph of dependence of signal response on hydrochlorothiazide concentration: *a* – regression graph; *b* – residual graph

5. Discussion of research results

Methods UPLC-MS detection began to gain more and more popularity. The use of a highly selective detector and chromatographic columns allows the development of rapid methods using a small volume of low-toxicity mobile phases (a mixture of 0.1 % formic acid and acetonitrile). Ramipril is non-sulfhydryl ACE inhibitor with antihypertensive effect. Hydrochlorothiazide is a diuretic that is often used to treat hypertension and oedema caused by fluid retention. Both ramipril and hydrochlorothiazide are resistant to heating in solution [5, 6], so it does not destroy when cleaning the equipment and can be determined in washing solutions. In the scientific literature, analytical methods for the determination of ramipril and hydrochlorothiazide in dosage forms are presented; however, no analytical method for controlling the cleaning of equipment is described. Previously, our scientific group had developed an HPLC method for the determination of ramipril in tablets, and we already had some developments in this direction, so we were interested in developing methods for monitoring the cleaning of equipment, which is important for routine pharmaceutical analysis [27]. The use of the MS detector was justified in this case for the purposes that we have planned. We have proposed column C18 Kinetex 2.1 mm×30 mm×1.7 μm) and mobile phase 0.1 % solution of formic acid in deionised water – acetonitrile (73:27) with flow rate 0.4 ml/min and column temperature 45 °C for determination of ramipril and mobile phase 0.1 % solution of formic acid in deionised water – acetonitrile (91.5:8.5) with flow rate 0.35 ml/min and column temperature 40 °C for determination of hydrochlorothiazide. In order to study the robustness in more detail, we performed validation on 2 detectors from different manufacturers (Agilent 6420 MS-detector and Waters Xevo TQD ACQUITY MS-detector). The proposed analytical methods have sufficient linearity, accuracy and precision. The sensitivity of the techniques was confirmed at the level of 0.0026 μg/ml. The techniques can be used in the concentration range of 0.0026–0.0255 μg/ml. We have fully presented the results of validation with calculation formulas, described its procedure and showed a new application of the technique for completely different purposes than determination in dosage forms, which is also no less important and cannot be ignored.

Practical Relevance. The proposed analytical methods can be used to determine the the residual quantities of ramipril and hydrochlorothiazide for controlling the cleaning of equipment.

Study limitations. The proposed methods can not be used to determine ramipril and hydrochlorothiazide in one single run.

Prospects for further research. The next research stage is planned to investigate the problems in the method development of the residual quantities of ramipril and hydrochlorothiazide for controlling the cleaning of equipment in the presence of other non-sulfhydryl ACE inhibitors.

6. Conclusions

Analytical methods for determining ramipril and hydrochlorothiazide in washing waters by UPLC-MS were developed and validated. The proposed analytical methods have sufficient linearity, accuracy, and precision. The developed methods are suitable for determining the residual amounts of ramipril and hydrochlorothiazide in the washing solution in the concentration range of 0.0026–0.0255 μg/ml. The methods can be used to control equipment cleaning for mono preparations and combined preparations.

Conflict of interests

The authors declare that they have no conflict of interest related to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Data availability

data will be made available on reasonable request

Use of artificial intelligence

The authors confirm they did not use artificial intelligence technologies when creating the current work.

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