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Research Article

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Quantitative estimation of new biologically active substances of derivated 4,5-dimethoxy-N-phenylanthranilic acids

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ABSTRACT

The method for quantitative estimation of 4,5-dimethoxy-N-phenylanthranilic acids was defined by the two-phase titration. Principle of the method consists in direct titration by alkali solution of two-phase system which is compounded of organic phase that encloses substances being analyzed, and of water phase that encloses indicator. The endpoint size of titration is defined according to decolorization of the water layer. The method is characterized by a high accuracy, simplicity, expressiveness. Relative error in the method does not exceed 0.5 %.

Key words: anthranilic, mefenamic, tolfenamic acids, method of two-phase titration.

INTRODUCTION

At the present time interest of scientific society to derivates of anthranilic acids as to prospective class of biologically active substances which have various activities as that follow: analgetic, anti-inflammatory, fungistatic, diuretic, anti-diuretic, sedative. Derivates of N-phenylanthranilic acid are widely applied as parent compounds for new substances synthesis, in particular, on the base of them the following effective medicines have been created (mefenamic, tolfenamic acids, antral, diftorant and others) [1-7].

Compounds of this class of derivatives according to the data in the European and British pharmacopeia [9, 10] are defined by the method of potentiometric titration in non-aqueous and mixed solvents. This method needs considerable loss of time and analyzed substance (200mg), and has low sensitiveness. That is why development of methods for quantitative estimation of 4,5-dimethoxy-N-phenylanthranilic acids is absolutely of a great practical interest.

The aim of research is developing of express method for quantitative estimation of 4,5-dimethoxy-N-phenylanthranilic acids by the method of two-phase titration in a system octanol – water.

EXPERIMENTAL SECTION

As objects of the research the 4,5-dimethoxy-N-phenylanthranilic acids were chosen (1-9), which structure are proved by the data of elmental, IR-, UV-, PMR – spectroscopy analysis and counter synthesis. For the first time synthesized compounds (1-9) show anti-inflammatory, analgetic, diuretic, antibacterial and fungistatic activity.

Equipment and reagents for quantitative estimation of mefenamic, tolfenamic and 4,5-dimethoxy-N-phenylanthranilic acids by the method of two-phase titration: microburette of class A (capacity – 5ml); glass-stoppered measuring flask (capacity – 100ml); n-octanol; 0.1% alcohol solution of thymolphthalein; sodium hydroxide (0.1M solution). All reagents and solutions were prepared according to demands of SPhU [8].

Potentiometric titration was conducted in mixed solvent «dioxane - water» (60 volume % dioxane) in ionomer I-160 with application of indicating glass electrode (ESP 43-07) and silver chloride (EVL - 1M4) reference electrode.

Method of quantitative estimation of 4,5-dimethoxy-2-(phenylamino) benzoic acid (1) by two-phase titration
The exact batch of 4,5-dimethoxy-2-(phenylamino)benzoic acid (0.1-0.15g) is being put into glass-stoppered measuring flask with capacity 100ml, then 20ml of octanol is being added and the batch is dissolving. Then 40ml of the distilled water and 8-10 drops of 0.1% alcoholic solution of phenolphthalein are being added. The titration is conducted by 0,1M solution of NaOH with an intensive agitation until the bright pink discoloration of the water layer. The compounds of 2-9, mefenamic and tolfenamic acids are being analyzed similarly (table 2).

Method of quantitative estimation of 4,5-dimethoxy-2-(phenylamine) benzoic acid (1) by potentiometric titration
The exact batch of 4,5-dimethoxy-2-(phenylamino)benzoic acid (0.1-0.15g) is being dissolving in 20ml of mixed solution of dioxane-water (60 volume % doixane) and is being ttitrated according to the potentiometric method by free-carbonated 0,1M solution of sodium hydroxide in the ionomer I-160 with application of indicating glass electrode (ESP 43-07) and silver chloride reference (EVL - 1M4) electrode. The endpoints are defined by the first derivative dependence E (mV) – f (V NaOH). The compounds of 2-9, mefenamic and tolfenamic acids are being analyzed similarly (table 2).

The quantitative calculation of the content of 4,5-dimethoxy-2-(phenylamino)benzoic (1-9), mefenamic and tolfenamic acids, %, is being performed by the formula:

$$\% = \frac{V \times K \times T \times 100}{m_S}$$

where V – volume of 0,1M solution of the sodium hydroxide, used for titration, ml; K – correction coefficient to molarity of the 0,1M sodium hydroxide solution; T – titre of the 0,1M sodium hydroxide solution according to the experimental compound, $g \times ml^{-1}$; m_S – mass of the experimental compound batch, g.

RESULTS AND DISCUSSION

We have developed the express method for quantitative estimation of 4,5-dimethoxy-N-phenylanthranilic acids. The method of two-phase titration with the presence of indicator that is not being extracted by organic solvents was taking as a basis. Principle of the method consists in direct titration by a standard water solution of sodium hydroxide of two-phase system which is compounded of the organic phase that encloses salvation of the experimental compound, and the water phase that encloses indicator. There is the extraction disbalance within the titration by sodium hydroxide solution and the sodium salt of 4,5-dimethoxy-N-phenylanthranilic acid rises into water phase. The endpoint size of titration is defined according to decolorization of the water layer.

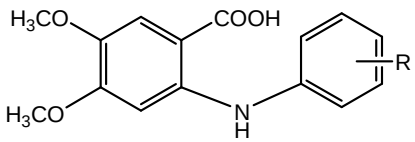
Table: Data of quantitative calculation of 4,5-dimethoxy-2-(phenylamino)benzoic acids by the method of two-phase titration with different acid-base indicators

Indicator	Compound batch, g	Defined, %	Metrological characteristics
0.04% alcoholic solution of m-cresol purple	0.5009 0.7152 0.8051 1.1951 1.5253	99.54 99.14 100.15 99.51 99.82	X = 99,63% S = 0,377 S_X = 0,1685 ΔX = 0,47 ε = 0,47%
0.1% alcoholic solution of phenolphthalein	0.1094 0.2020 0.6088 0.9114 1.5017	100.08 99.55 100.30 99.82 100.18	X = 99,99% S = 0,3011 S_X = 0,135 ΔX = 0,37 ε = 0,37%
0.1% alcoholic solution of thymolphthalein	0.1237 0.1410 0.5012 0.9014 1.4012	100.28 99.76 100.41 100.26 99.84	X = 100,11% S = 0,290 S_X = 0,130 ΔX = 0,67 ε = 0,67%

The optimal conditions for two-phase titration of non-described in literature 4,5-dimethoxy-N-phenylanthranilic acids were determined. N-octanol is being used as an organic solvent which solves well the experimental compounds (1-9). The choice of N-octanol as a solvent is caused both by a good solvability, and a usage of the octanol-water mixture as a model one

for evaluation of lipophilic activity of biologically active substances. The experimentally founded correlation of volume in the water and organic phases equal 2:1. As indicators 0.1% alcoholic solution of phenolphthalein, 0.04% alcoholic solution of m-cresol purple and 0.1% alcoholic solution of thymolphthalein can be used. According to data in the table 1 it is evident that the alcoholic solution of phenolphthalein is the most acceptable indicator because the ≈ 0.1g batch of the experimental substances is enough while this solution is being used (table 1).

Table 2: Data of quantitative calculation of substituted 4,5-dimethoxy-2-(phenylamino)benzoic acids by the method of two-phase and potentiometric titration



Compound	Two-phase titration			Potentiometric titration		
R	Batch, g	Defined, %	Metrological characteristics	Batch, g	Defined, %	Metrological characteristics
1	2	3	4	5	6	7
H	0.1028 0.1039 0.1104 0.1088 0.1092	99.79 100.26 100.01 99.93 100.21	$\bar{x}$ = 100,04% S = 0,195 $S_{\bar{x}}$ = 0,087 $\Delta\bar{x}$ = 0,24 $\bar{\varepsilon}$ = 0,24%	0.1159 0.1172 0.1138 0.1212 0.1239	100.00 100.68 100.00 100.11 99.86	$\bar{x}$ = 100,01% S = 0,106 $S_{\bar{x}}$ = 0,047 $\Delta\bar{x}$ = 0,13 $\bar{\varepsilon}$ = 0,13%
2'-CH_3	0.1067 0.1071 0.1095 0.1055 0.1120	99.71 100.60 100.24 99.51 100.44	$\bar{x}$ = 100,10% S = 0,503 $S_{\bar{x}}$ = 0,225 $\Delta\bar{x}$ = 0,62 $\bar{\varepsilon}$ = 0,62%	0.1094 0.1145 0.1169 0.1209 0.1214	99.99 100.05 100.01 99.94 99.98	$\bar{x}$ = 100,01% S = 0,0612 $S_{\bar{x}}$ = 0,0274 $\Delta\bar{x}$ = 0,08 $\bar{\varepsilon}$ = 0,08%
4'-CH_3	0.1085 0.1074 0.1057 0.1052 0.1045	100.31 100.44 100.34 99.51 99.04	$\bar{x}$ = 99,93% S = 0,636 $S_{\bar{x}}$ = 0,284 $\Delta\bar{x}$ = 0,67 $\bar{\varepsilon}$ = 0,67%	0.1153 0.1077 0.1105 0.1201 0.1108	99.89 99.40 100.30 99.51 99.29	$\bar{x}$ = 99,68% S = 0,404 $S_{\bar{x}}$ = 0,181 $\Delta\bar{x}$ = 0,09 $\bar{\varepsilon}$ = 0,09%
3',4'$(CH_3)_2$	0.1093 0.1102 0.1192 0.1220 0.1399	99.99 100.08 99.78 99.45 99.46	$\bar{x}$ = 99,74% S = 0,281 $S_{\bar{x}}$ = 0,125 $\Delta\bar{x}$ = 0,35 $\bar{\varepsilon}$ = 0,35%	0.1132 0.1124 0.1120 0.1025 0.9991	98.87 99.24 98.96 99.41 99.61	$\bar{x}$ = 99,22% S = 0,3070 $S_{\bar{x}}$ = 0,1373 $\Delta\bar{x}$ = 0,38 $\bar{\varepsilon}$ = 0,38%
4'-OCH_3	0.1032 0.1018 0.1012 0.1039 0.1042	99.77 100.02 100.47 99.86 100.39	$\bar{x}$ = 100,10% S = 0,389 $S_{\bar{x}}$ = 0,165 $\Delta\bar{x}$ = 0,46 $\bar{\varepsilon}$ = 0,46%	0.1104 0.1029 0.1051 0.1037 0.1049	100.05 100.07 100.63 100.09 99.81	$\bar{x}$ = 100,1% S = 0,106 $S_{\bar{x}}$ = 0,047 $\Delta\bar{x}$ = 0,13 $\bar{\varepsilon}$ = 0,13%
1	2	3	4	5	6	7
4'-OC_2H_5	0.1016 0.1024 0.1008 0.1024 0.1020	100.33 100.44 99.45 100.30 99.53	$\bar{x}$ = 100,01% S = 0,476 $S_{\bar{x}}$ = 0,213 $\Delta\bar{x}$ = 0,59 $\bar{\varepsilon}$ = 0,59%	0.1092 0.1005 0.1038 0.1028 0.1031	99.56 99.69 100.03 99.99 99.80	$\bar{x}$ = 99,81% S = 0,187 $S_{\bar{x}}$ = 0,083 $\Delta\bar{x}$ = 0,02 $\bar{\varepsilon}$ = 0,02%

4'-OC_3H_7	0.1033 0.1059 0.1022 0.1030 0.1045	99.98 99.80 100.01 99.90 99.71	$\bar{x}$ = 99,88% S = 0,130 $S_{\bar{x}}$ = 0,058 $\Delta\bar{x}$ = 0,16 $\bar{\varepsilon}$ = 0,16%	0.1045 0.1024 0.1029 0.1017 0.1021	99.95 99.80 100.03 100.15 100.45	$\bar{x}$ = 100,08% S = 0,245 $S_{\bar{x}}$ = 0,109 $\Delta\bar{x}$ = 0,30 $\bar{\varepsilon}$ = 0,30%
4'-Br	0.1102 0.1084 0.1085 0.1062 0.1050	99.76 98.56 100.10 99.94 98.70	$\bar{x}$ = 99,21% S = 0,713 $S_{\bar{x}}$ = 0,318 $\Delta\bar{x}$ = 0,89 $\bar{\varepsilon}$ = 0,89%	0.1036 0.1025 0.1028 0.1015 0.1019	100.04 99.02 99.63 99.07 99.90	$\bar{x}$ = 99,53% S = 0,542 $S_{\bar{x}}$ = 0,242 $\Delta\bar{x}$ = 0,67 $\bar{\varepsilon}$ = 0,67%
2'-Cl	0.1058 0.1139 0.1024 0.1011 0.1059	99.50 99.03 99.00 98.80 99.82	$\bar{x}$ = 99,29% S = 0,407 $S_{\bar{x}}$ = 0,182 $\Delta\bar{x}$ = 0,51 $\bar{\varepsilon}$ = 0,51%	0.1058 0.1074 0.1085 0.1108 0.1123	100.50 100.15 100.30 99.82 99.84	$\bar{x}$ = 100,12% S = 0,319 $S_{\bar{x}}$ = 0,043 $\Delta\bar{x}$ = 0,40 $\bar{\varepsilon}$ = 0,40%
Mefenamic acid	0.1205 0.1131 0.1204 0.1056 0.1076	100.17 100.35 99.85 99.77 100.41	$\bar{x}$ = 100,11% S = 0,290 $S_{\bar{x}}$ = 0,130 $\Delta\bar{x}$ = 0,33 $\bar{\varepsilon}$ = 0,33%	0.1300 0.1198 0.1242 0.1238 0.1209	99.44 98.83 99.79 99.52 99.61	$\bar{x}$ = 99,44% S = 0,359 $S_{\bar{x}}$ = 0,160 $\Delta\bar{x}$ = 0,44 $\bar{\varepsilon}$ = 0,44%
1	2	3	4	5	6	7
Tolfenamic acid	0.1062 0.1123 0.1071 0.1075 0.1090	100.04 100.55 99.84 100.10 100.06	$\bar{x}$ = 100,13% S = 0,229 $S_{\bar{x}}$ = 0,102 $\Delta\bar{x}$ = 0,28 $\bar{\varepsilon}$ = 0,28%	0.1059 0.1062 0.1099 0.1101 0.1044	99.95 99.33 99.62 99.50 99.22	$\bar{x}$ = 99,52% S = 0,275 $S_{\bar{x}}$ = 0,123 $\Delta\bar{x}$ = 0,34 $\bar{\varepsilon}$ = 0,34%

The comparable data of the mefenamic, tolfenamic and 4,5-dimethoxy-N-phenylanthranilic acids (1-9) estimation by the method of two-phase titration and well-known potentiometric method in the mixed solvent dioxane-water (60 vol. % dioxane) are presented in the table 2.

The obtained results of quantitative estimation by the two-phase titration are characterized by the accuracy and representativity. The relative uncertainty of the average result by this method does not exceed 0.5%. The method developed is expressive, easy to use, and reliable. These characteristics differ this method advantageously from the method of potentiometric titration.

Nature of substitutes and their location in anthranilic fragments of 4,5-dimethoxy-N-phenylanthranilic acids do not affect on the quantitative estimation results.

CONCLUSION

1. The express method for quantitative estimation of 4,5-dimethoxy-N-phenylanthranilic acids by the two-phase titration in a system octanol-water has been defined.

2. The optimal conditions for two-phase titration in a system octanol-water have been determined, the indicator has been chosen, which application let to use less amount of the experimental substance. The method developed is characterized by simplicity, expressiveness, reliability and a high enough accuracy.

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