DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR META-IODOBENZYLGUANIDINE SULFATE RADIOLABELED WITH ^{127}I AND ^{131}I

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Methods for determining the quantitative contents of *meta*-iodobenzylguanidine (MIBG) and iodide ion, the chemical purity of MIBG, and the radiochemical purity of [^{131}I]-MIBG using HPLC with spectrophotometric and radiometric detection were developed. The detection limits were $50 – 250$ μg/mL for MIBG, $0.78 – 58.5$ μg/mL for NaI, and $300 – 2250$ kBq for [^{131}I]-MIBG. Validation of the methods confirmed that their characteristics met the specified requirements.

Keywords: *meta*-iodobenzylguanidine, radiopharmaceutical, HPLC, RP, MIBG.

meta-Iodobenzylguanidinium sulfate (Fig. 1) labeled with ^{131}I ([^{131}I]-MIBG) is widely used in medical practice for diagnosis and therapy of malignant neoplasms [1, 2].

[^{131}I]-MIBG is a structural analog of noradrenaline and accumulates selectively after i.v. injection in tissues with high adrenergic innervations (stimulated by adrenaline and noradrenaline), especially in neuroectodermal tumors.

The preparation possesses clearly pronounced affinity for the target organ and damaged tissues and accumulates mainly in excretory organs, salivary glands, adrenals, lungs, and spleen [2].

The main processing impurities that should be controlled are the MIBG precursor *meta*-iodobenzylammonium chloride (MIBA) and the medical radioisotope ^{131}I used in the synthesis. International pharmacopoeias establish the following limits on impurity contents: MIBA, $\leq 1\%$; ^{131}I, $\leq 5\%$ for diagnosis and $\leq 7\%$ for therapy. The present article focused on method development for controlling them.

Chemical purity and content of MIBG. Nonaqueous titration and HPLC can be used to determine the quantitative content of main ingredient. Each method has its pluses and minuses. HPLC is a relative method that must be calibrated using a reference standard of the drug substance, which is not required for titration because the titration is measured using

common compounds. The absoluteness of the method allows the compound concentration to be determined immediately without using calibration curves and mathematical models. However, nonaqueous titration places high demands on the experimental set up and the quality of used reagents. Also, impurities that affect the analytical results must be masked. Mixtures of aromatic amines were titrated before [3 – 5]. However, HPLC can determine the content and chemical purity in one analysis.

HPLC methods for determining the contents of main ingredients and chemical purity are known from European (EuPha) [6], US (USP) [7], and International Pharmacopoeias [8] and the scientific literature [9 – 11]. However, they had different drawbacks in practice so we decided to develop our own.

Radiochemical purity can be determined by planar chromatography and electrophoresis with detection by β- or γ-spectrometry and HPLC with β- and γ-radiation detectors. The accuracy of planar chromatography methods depends

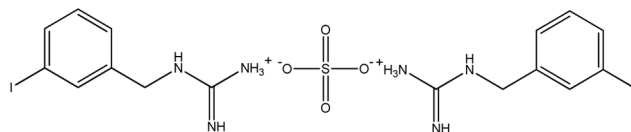**Fig. 1.** *meta*-Iodobenzylguanidinium sulfate.

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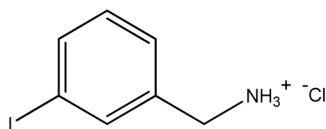* e-mail: commander-scaramouche@hotmail.com

Fig. 2. *meta*-Iodobenzylammonium chloride.

Fig. 3. UV spectrum of MIBG.

strongly on the collimator slit width and geometry for β- and γ-scanners and on the distance from the plate to the collimator or on the size of the plate tracks and paper, the activity of which is measured using a spectrometer. Such problems are not observed for HPLC. However, the Raytest Ramona Star detector used by us gave a flattened and broadened analyte peak for γ-rays because of the measurement cell geometry and hindered rapid analyses. The lower background of β-particles (as compared with γ-photons) was considered in selecting an analytical method and detector. Because of this and an existing developed method for determining the chemical purity and content of MIBG, HPLC with β-spectrometric detection was selected.

EXPERIMENTAL PART

Samples of MIBG drug substance, finished preparation [131I]-MIBG, and processing impurities MIBA, NaI, and Na131I were used for the research.

A pharmacopoeial standard (EuPha: Iobenguane sulfate, cat. Code 10328000) was used to develop the method for determining MIBG content. The standard for developing a method to determine the content of iodide ions was working reference standard (WRS) NaI (analytically pure, Reakhim). The standard for developing a method to determine the radiochemical purity of [131I]-MIBG was a certified Na131I solution (KPRI).
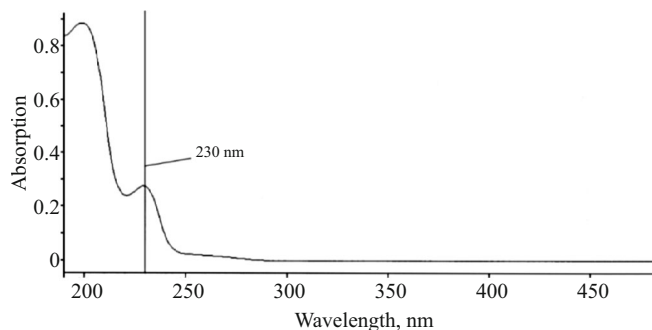
Solutions of actual samples were prepared from MIBG and MIBA (KPRI).

The studies used a Shimadzu LC-20AD HPLC with a Shimadzu SPD-20A UV detector and a Shimadzu LC-20AT HPLC (Shimadzu Corp., Japan) with a Raytest Ramona Star radiometric detector (Elysia-Raytest GmbH, Germany) with a cell for detecting β-radiation.

The ranges of studied concentrations and activities were "cold" MIBG, 50 – 250 μg/mL; NaI, 0.78 – 58.5 μg/mL; and [131I]-MIBG + Na131I, 300 – 2250 kBq.

All validation tests were planned, set up, and executed in compliance with State Pharmacopoeia XIVth Ed. (SP XIV) [12], RMG 61-2010 "Accuracy, trueness, and precision measures of the procedures for quantitative chemical analysis" (RMG 61-2010) [13]. Calculations were carried out according to SP XIV, RMG 61-2010, MI 2083-90 "Indirect measurements. Determination of measurement results and estimation of their errors" (MI 2083-90) [14] and GOST R 8.736-2011 "Multiple direct measurements. Methods for processing measurement results" [15]. Dixon and Grubbs criteria were used to check for outliers; Fisher criterion, the model adequacy; Pearson criterion, correlation functions.
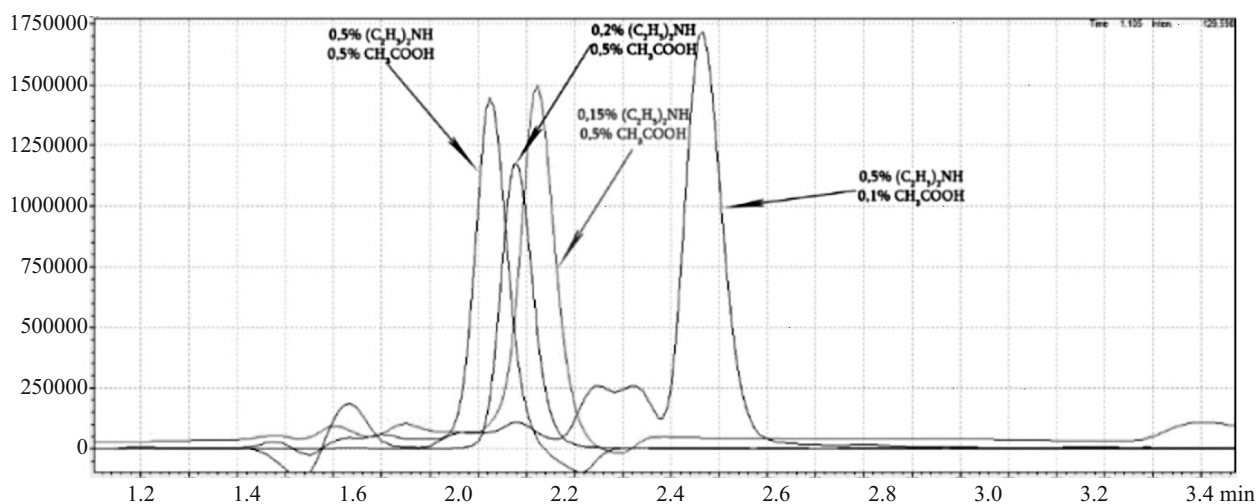

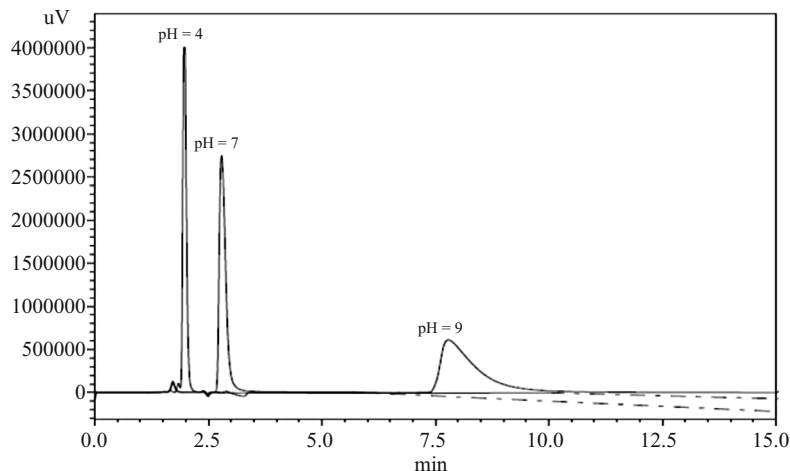Fig. 4. Effect of modifiers on separation of MIBG and MIBA.

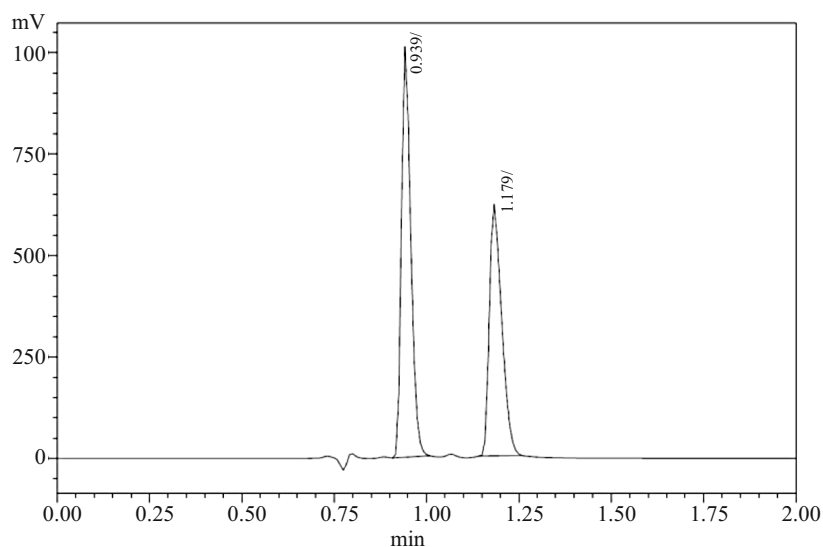**Fig. 5.** Effect of pH on broadening and retention time of the peak.

Fig. 6. Chromatogram for separation of a mixture of MIBA (0.939 min) and MIBG (1.179 min).

The significance of coefficients in regression equations was checked using the Student and p-criteria. Critical values of Student and Fisher statistical distributions and Grubbs criteria were taken from *Tables of Mathematical Statistics* [16]

TABLE 1. Statistical and Validation Parameters of the Methods

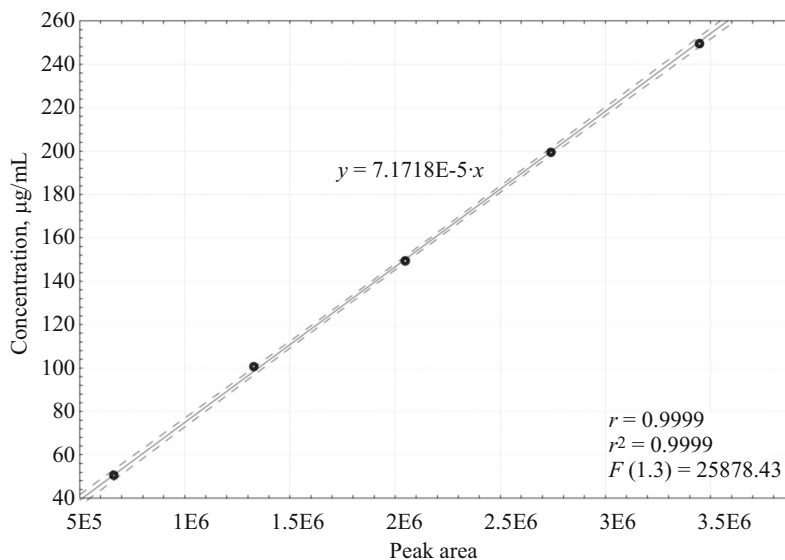| Analyte	Concentration range, µg/mL	Regression equation	Statistical parameters	Validation criteria
MIBG sulfate	50 – 250	$C = 7.17 \times 10^{-5} \times x$	$r = 0.9999$ $r^2 = 0.9999$ $F(1.3) = 25878$	Precision = 95% CI [– 2.41, 2.41%] Trueness = 2.66% Accuracy = 4.76%
Sodium iodide	0.78 – 58.5	$C = 1.51 \times 10^{-5} \times x$	$r = 0.9997$ $r^2 = 0.9994$ $F(1.6) = 9239$	Precision = 95% CI [– 3.19, 3.19%] Trueness = 1.6% Accuracy = 3.92%

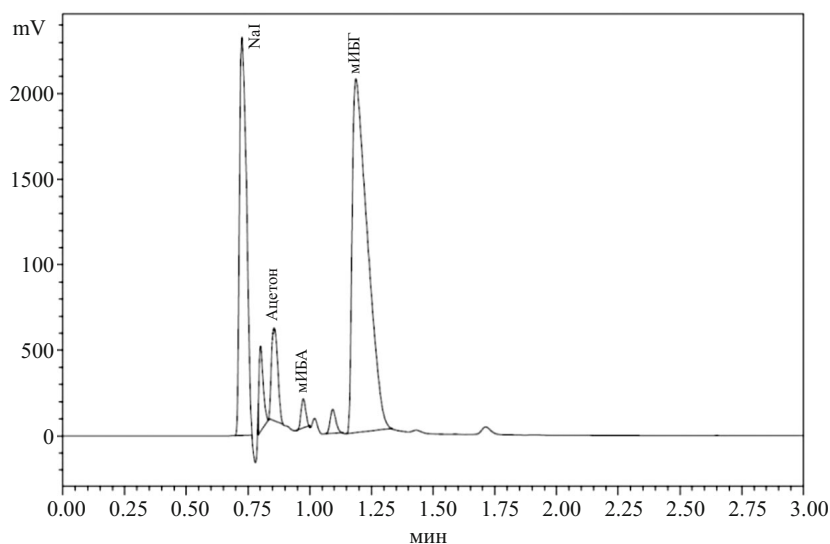**Fig. 7.** Plot of MIBG sulfate concentration vs. peak area.

Fig. 8. Chromatogram of a mixture of NaI, Me$_2$CO, MIBA, MIBG, and unknown impurities after sample irradiation ($\lambda = 230$ nm).

and GOST R ISO 5725-2-2002 "Accuracy (trueness and precision) of measurement methods and results" [17].

RESULTS AND DISCUSSION

Review of existing analytical methods. The pharmacopoeial monograph in the EuPha 9.0 proposes using wavelength 254 nm for detection. Research showed that the absorption maximum was located near 230 nm (Fig. 3). The mobile phase composition with a pH of 8 raised issues because pH $\leq$ 7.5 is critical if silica gel without a grafted phase is used. The USP does not justify the choice of rather large-grained sorbent (10 μm) and the semi-preparative col-

umn size. The phosphoric acid in the mobile phase limits the ability to use the method with evaporative and aerosol detectors. Poor reproducibility of analytical results was observed during selection of the separation conditions. The EuPha method gave a highly flattened asymmetric analyte peak that eluted much later than the regulated time, i.e., 13 min using a Merck Purospher STAR Si column (5μ, 125 $\times$ 4 mm) vs. 7 min using a silica gel column (5μ, 250 $\times$ 4 mm). MIBA and MIBG in the tested mixture could not be separated using the USP method.

Separation process. The roles of the mobile-phase modifiers were defined during development of the analytical method. HOAc accelerated desorption of the compound from
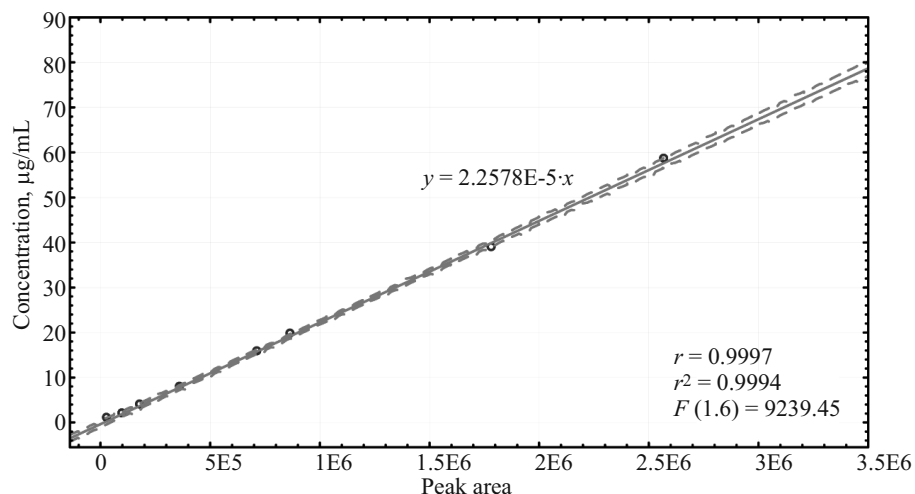
Fig. 9. Plot of NaI concentration vs. peak area.

the column. Et_2NH enhanced adsorption and the peak asymmetry diminished (Fig. 4).

MIBG could be expected to have strongly basic properties because of the guanidine moiety in the cation. However, studies of MIBG solubility found that the benzene ring contributed much more to the chemical properties than the guanidine group. Small amounts of added acid and amine (≤ 0.5 mL/100 mL of eluent) had positive effects (decreased asymmetry, narrower width, shorter retention time). Addition of 1 mL/100 mL of eluent and greater caused a slight reduction in the separation parameters, possibly because of the increased ionic strength of the solution and increased competition of the analyte and solvent in the eluent layer on the sorbent particle surface [18].

The effect of pH on the distribution of MIBG in the mobile and stationary phases was studied. The retention time of the salt was shorter in acidic solution; longer, in basic. The dependences between pH and modifier concentration and separation parameters were nonlinear (Fig. 5).

Selection and optimization of MIBG analytical conditions. Validation and study of method applicability limits. The research and development on the separation conditions for determining the MIBG content in several batches of samples found the optimal conditions to be:

Column	Merck ChromoLith High Resolution RP-18 100×4.6 mm
Mobile phase	$25\%_{v/v}$ CH_3CN: $75\%_{v/v}$ H_2O : $0.2\%_{v/v}$ CH_3COOH : $0.1\%_{v/v}$ $(C_2H_5)_2NH$
Flow rate	2.0 mL/min
Temperature	45°C
Detector	spectrophotometric, 230 nm
Sample volume	20 μL
Analysis time	2 min

Validation of the process found that MIBG concentrations in the range 50 – 250 μg/mL were described most accurately by the equation $C = 7.17 \times 10^{-5} \times x$, where C is the MIBG concentration (μg/mL); x, peak area. The direct dependence of the concentration on peak area had a high correlation coefficient ($r = 0.9999$). The regression model described the obtained results with a determination coefficient of 0.9999 (Figs. 6 and 7). Table 1 presents the precision as the confidence interval limits; the trueness as the standard deviation of unexcluded systematic error in the analytical method, and the accuracy as the standard deviation of the uncertainty in the analytical results (total systematic and random errors).

TABLE 2. Statistical Criteria and Adequacy of Regression Models of Linearity

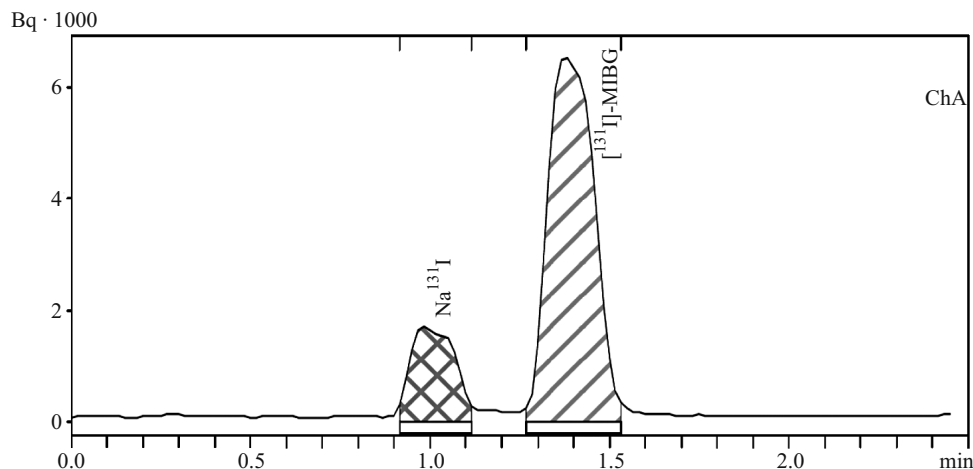| Dependence	Regression equation	Statistical criteria	Statistical significance of model
Sample activity – [^{131}I]-MIBG peak area	$A = 0.0245x$	$r = 0.9962$, $r^2 = 0.9924$ $F(1.3) = 391.51$	Significant
Sample activity – Na^{131}I peak area	$A = 0.1045x$	$r = 0.9896$, $r^2 = 0.9793$ $F(1.3) = 142.12$	Significant
Radiochemical purity – sample activity	$RCP = 0.7275 + 3.51 \times 10^{-5} \times x$	$r = 0.8456$, $r^2 = 0.7150$ $F(1.3) = 7.53$	Not significant

Fig. 10. Chromatogram of a mixture of Na^{131}I and [^{131}I]-MIBG.

The dependence of NaI concentrations in the range 0.78 – 58.5 µg/mL on peak area obeyed the equation: $C = 1.51 \times 10^{-5} \times x$, where C is the NaI concentration (µg/mL); x, peak area. The direct dependence of concentration on peak area had a high correlation coefficient ($r = 0.9997$) (Fig. 9). A regression model described the results with a determination coefficient of 0.9994. Table 1 presents the precision, trueness, and accuracy.

The radiation stability of MIBG sulfate was studied to determine if the proposed method could be used with MIBG in the presence of various radioprotectors. The method was highly selective and could be used to separate mixtures of MIBG, iodides, Me$_2$CO, methylethylketone, EtOH, and *p*-hydroxybenzoic acid (Fig. 8).

Optimization and validation of the method for determining MIBG radiochemical purity. The analytical conditions had to be slightly altered to determine MIBG radiochemical purity because of specifics of the apparatus and the operation of the radiometric detector:

Column	Merck ChromoLith High Resolution RP-18 100 × 4.6 mm
Mobile phase	33%$_{v/v}$ CH$_3$CN : 66%$_{v/v}$ H$_2$O : 0.2%$_{v/v}$ CH$_3$COOH
Flow rate	1.5 mL/min
Temperature	40°C
Detector	β-spectrometric, channels 0 – 1023
Sample volume	20 µL
Analysis time	3 min

The WRS for [^{131}I]-MIBG was an impurity (Na^{131}I) that was detected by γ-spectrometry because a reference standard could not be obtained. The method of additions using a standard impurity as described in RMG 61-2010 was used to evaluate the quality indicators. A sample with specific activity 768 kBq/mL and an additive with Na^{131}I = 127.6 kBq/mL

were used (Fig. 10). Errors were calculated according to MI 2083-90 and GOST R 8.736-2011. The indicator values in percent of the radiochemical purity were precision 2.2%; trueness, 2.93; accuracy, 6.14.

Linearity was experimentally demonstrated in samples with identical radiochemical purity but different specific activities in the range 300 – 2250 kBq/mL. The first stage of statistical processing consisted of reproduction of the regression equation, calculation of statistical criteria for component peak areas as functions of sample activity, and confirmation of the adequacy of the constructed model. The second stage included reproduction of the linear regression equation describing the radiochemical purity as a function of added sample activity. Table 2 presents the values for the statistical criteria and regression models.

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