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Synthesis and Quantification of 2-(Diethylamino)-*N*-(2-methylphenyl)acetamide Nitrate

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Abstract—A procedure for the synthesis of 2-(diethylamino)-*N*-(2-methylphenyl)acetamide nitrate (*monomecaine*) exhibiting a pronounced antiarrhythmic activity has been optimized. A procedure for its identification and quality control of the drug substance by HPLC has been developed, and the limit of detection of *o*-toluidine impurity therein has been estimated at 0.02%. Procedures for HLPC and extraction–titration quantification of monomekain in the drug substance have been proposed and validated. The obtained relative standard deviation (RSD) indicates good specificity, linearity, and precision of the developed procedures.

Keywords: monomecaine, HPLC, extraction titration, validation.

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Cardiovascular diseases constitute one of the leading causes of death over the world. According to statistical data, each thirteenth citizen of the Russian Federation suffers from one or another cardiac or vascular pathology, and the mortality caused by cardiovascular diseases amounts to 53% of all deaths in Russia. These diseases are generally accompanied by arrhythmia, i.e., heart rhythm rate disorders which could aggravate the underlying disease. Although the series of available antiarrhythmic drugs is sufficiently large, many of them cause undesirable side effects; therefore, development and introduction into medical practice of new antiarrhythmic agents constitute an important problem [1, 2]. Various compounds are being studied for this purpose. The most widely used anesthetics and antiarrhythmics are represented by derivatives of *N*-phenylacetamide and aromatic carboxylic acid amides [3–11], lidocaine being the most known [12]. Of particular interest are *N*,*N*-diethylaminoacetic acid *o*-toluidide [2-(diethylamino)-*N*-(2-methylphenyl)acetamide] salts with inorganic acids. A procedure for the preparation of 2-(diethylamino)-*N*-(2-methylphenyl)acetamide hydrochloride was described in [9, 13]. Later on, 2-(diethylamino)-*N*-(2-methylphenyl)acetamide salts with HBr, HI, and H_3PO_4 [9] and *N*-(2-methylphenyl)-2-(morpholin-4-yl)-acetamide salt with salicylic acid [10] were synthesized. Their antiarrhythmic activity was evaluated, and prospects of further studies aimed at introducing them into medical practice have been demonstrated. 2-(Diethylamino)-*N*-(2-methylphenyl)acetamide nitrate arbitrarily called *monomecaine* is promising as a potential antiarrhythmic drug. It exhibited the highest activity among the above listed 2-(diethylamino)-*N*-(2-methylphenyl)-acetamide salts [9–11] (Table 1), and it can therefore be regarded as a potential antiarrhythmic.

Preclinical trials of a chemical compound require its standardization which includes development of a procedure for its preparation ensuring high purity of the product, efficient methods for quality evaluation of both drug substance and dosage forms, and procedures for its determination in biological media. No quality control methods acceptable for the development of a state pharmacopeial specification for a dosage form have been reported. Therefore, the goal of the present work was to optimize the procedure for the synthesis of monomecaine and develop procedures for its identification and purity evaluation by HPLC and for

Table 1. Antiarrhythmic activity of 2-(diethylamino)-*N*-(2-methylphenyl)acetamide salts and lidocaine

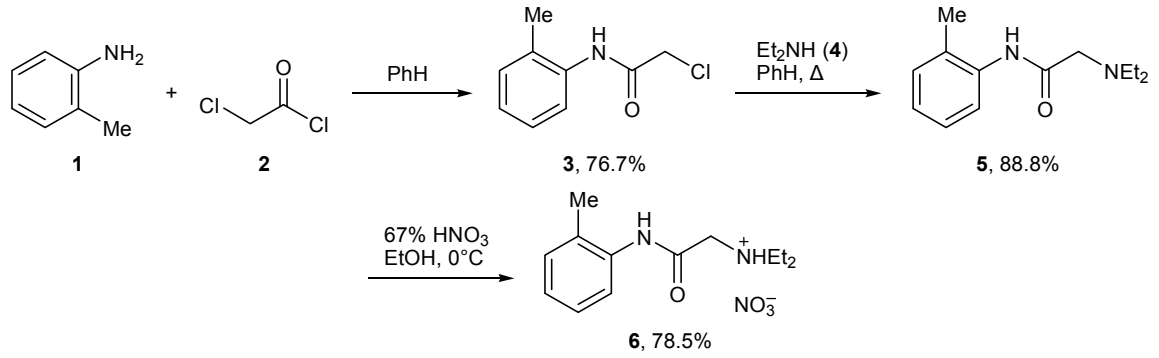
Compound	LD_{50}, mg/kg	ED_{50}, mg/kg	Antiarrhythmic index LD_{50}/ED_{50}
2-(Diethylamino)-*N*-(2-methylphenyl)acetamide nitrate (monomecaine)	65.0 (56–75)	1.4 (1.2–1.7)	46.4
2-(Diethylamino)-*N*-(2-methylphenyl)acetamide hydrochloride	46.0 (33–60)	1.4 (1.1–1.7)	32.8
2-(Diethylamino)-*N*-(2-methylphenyl)acetamide hydrobromide	81.5 (71–84)	17.8 (15–21)	4.6
2-(Diethylamino)-*N*-(2-methylphenyl)acetamide hydroiodide	60.0 (48–74)	35.5 (29–42)	1.7
2-(Diethylamino)-*N*-(2-methylphenyl)acetamide dihydrogen phosphate	44.7 (37–59)	20.5 (18–24)	2.2
Lidocaine	39.5 (34–45)	7.7 (6–9)	5.1

quantification in laboratory batches of the drug substance by HPLC and extraction–titration method.

The key precursor to monomecaine, 2-(diethylamino)-*N*-(2-methylphenyl)acetamide, was previously synthesized by acylation of *o*-toluidine with chloroacetyl chloride in glacial acetic acid or acetone according to a two-step procedure [13]. We improved this method by carrying out the reaction in one step in acetone or benzene by adding 1, 1.5, or 2 equiv of chloroacetyl chloride (**2**) to *o*-toluidine (**1**) (Scheme 1). The best yield was obtained in benzene (dried over metallic sodium) using 1.5 equiv of **2**. The acylation was complete almost instantaneously; a white solid precipitated immediately after addition of chloroacetyl chloride to a solution of *o*-toluidine **1**, and no initial amine was detected in the reaction mixture. The subsequent reaction of anilide **3** with diethylamine (**4**) gave 2-(diethylamino)-*N*-(2-methylphenyl)acetamide (**5**) which was dissolved in ethanol and treated with concentrated nitric acid at 0°C to obtain monomecaine (**6**). The structure and purity of the isolated compounds were confirmed by elemental analyses and ^{1}H NMR, HPLC, and TLC data.

In keeping with modern requirements, chromatographic methods are important for drug quality control at different stages of analysis of drug substance and dosage forms [14–16]. We propose HPLC as a method for quality control of monomecaine to be included in a pharmacopeial article project. Taking into account that monomecaine and possible related impurities [*o*-toluidine (**1**) and 2-chloro-*N*-(2-methylphenyl)acetamide (**3**)] are polar compounds, the use of reversed-phase HPLC seemed to be preferred. Initially, HPLC conditions for the determination of monomecaine and compounds **1** and **3** were found (Fig. 1). It was shown that neither monomecaine nor 2-chloro-*N*-(2-methylphenyl)acetamide synthesized according to the proposed procedure contained related impurities. With the

Scheme 1.



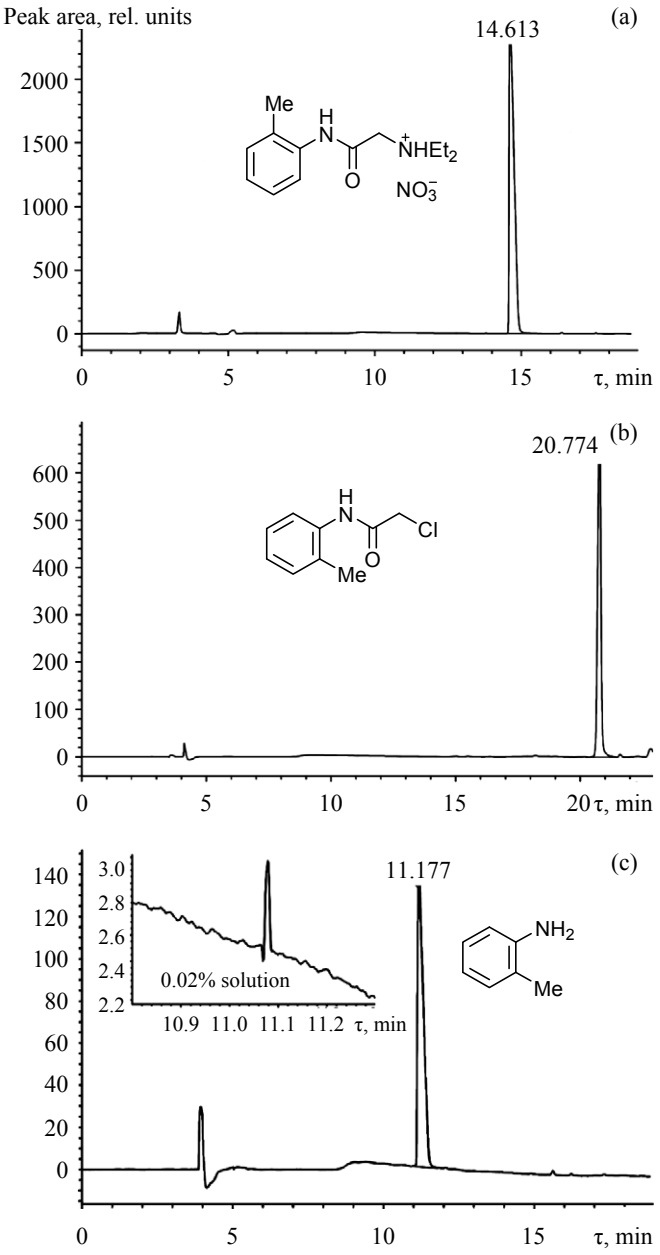


Fig. 1. HPLC chromatograms of (a) monomecaine (**6**), (b) 2-chloro-*N*-(2-methylphenyl)acetamide (**3**), and (c) 1% and 0.02% (in the insert) solutions of *o*-toluidine (**1**).

goal of estimating the *o*-toluidine detection limit in samples of **6**, model solutions of **6** containing 0.01 and 0.02% of **1** were prepared and analyzed.

The high resolution of peaks of **6** and **1** (R = 8.8) allowed us to successfully solve the problem. The peak of **1** on the chromatogram of the first solution (0.01% *o*-toluidine) insignificantly exceeded the background level, whereas the signal-to-noise (S/N) ratio for a 0.02% solution of **1** was 10.4 (Fig. 1c). Thus, in accordance with OFS.1.1.0012.15 [14], the limit of detection of *o*-toluidine in monomecaine substance was estimated at 0.02%. Analysis of four laboratory monomecaine batches according to the developed procedure showed the absence of *o*-toluidine in the examined samples. Taking into account considerable difference in the retention times of monomecaine and related impurities, the proposed method can also be used for the identification of the former.

The use of HPLC for the quantification of monomecaine in the drug substance and possible dosage forms developed at the preclinical trial stage, as well as for studying its metabolism and pharmacokinetic, also seemed reasonable. Validation is necessary for the HPLC procedure to be used in pharmacokinetic studies of drug dosage forms or included in a pharmacopeial article for a dosage form [15–17]. For this purpose, HPLC analyses of monomecaine solutions with different concentrations and a blank solution (containing no monomecaine) were performed (Fig. 2). The procedure was validated with respect to the following parameters: selectivity, linearity, analytical range, precision, and reproducibility.

The selectivity was estimated by analyzing the solvent used for the preparation of monomecaine solutions. The obtained chromatogram showed no peaks with a retention time corresponding to that of monomecaine (Fig. 2c). The linearity was checked by plotting a calibration curve (Fig. 3) for solutions containing 0.001, 0.0107, 0.0535, 0.214, and 1.07 mg/mL of monomecaine. Each solution was analyzed in triplicate. A linear dependence was observed between the chromatographic peak area and monomecaine concentration in the given range. The linear dependence is described by the equation $y = 26859.6\,x + 131$, and the correlation coefficient is 0.9997. The analytical range was defined as 0.8–1.2 mg/mL.

The precision of the proposed procedure was determined by statistical processing of the results of determination of monomecaine concentration in 3 model solutions containing 0.80, 1.00, and 1.20 mg/mL of the substrate on different working days. Standard deviations (S_δ) and relative standard deviations ($S_{\delta av}$) were calculated; the latter did not exceed 3% (Table 2), indicating acceptable precision of monomecaine quantification by the proposed procedure.

The reproducibility was estimated by statistical treatment of the results of analysis of samples from three laboratory batches of the drug substance. Student's t tests were calculated for the number of degrees of freedom $f = L - 1 = 4$ and a confidence

probability P of 0.95. In all cases, the calculated t values were lower than the reference value, indicating that the bias is insignificant with respect to random dispersion; therefore, it was assumed to be zero, and the relative error for the mean value (ε_m) did not exceed 3% (Table 3). Thus, the procedure gives accurate and reproducible results.

We can conclude that the proposed reversed-phase HPLC procedure for quantification of monomecaine is characterized by acceptable specificity, linearity, precision, and reproducibility; therefore, it can be used for analytical purposes.

We previously described another procedure for the quantification of monomecaine by nonaqueous acidimetry [18]. Herein, we propose one more method based on two-phase extraction–titration using sodium lauryl sulfate. Monomecaine is a salt formed by an organic base; therefore, it forms an associate with the titrant, which is extracted into organic phase (chloroform). The main factors affecting the extraction process are pH of the aqueous phase, aqueous and organic phase volume ratio, and selected indicator. The optimal conditions were found by varying one parameter, the other parameters being maintained constant. The optimal pH value was in the range 2.18–2.60, which corresponded to an initial HCl concentration of 0.02 M; the volume ratio of the aqueous and organic phases was estimated at 1:1 and 1:2; and a 5:1 mixture of Butter Yellow and Methylene Blue was used as an indicator (a sharp color change from light yellow to blue was observed at the equivalence point).

This procedure was also validated with respect to linearity of the results, analytical range, precision, and reproducibility. Statistical treatment of the results of quantification of monomecaine at 7 concentration levels gave a strictly linear relation between the concentration and equivalent titrant volume, which is given by the equation $y = 355.5\,x - 0.028$ with a correlation coefficient of 0.9999. The analytical range was defined as 80 to 120% of the concentration 0.03 mg/mL taken as 100%.

The precision of the extraction–titration method was estimated by statistical processing of the results of quantitation of monomecaine at three concentration levels within the analytical range. The relative standard deviation ($S_{\delta av}$) did not exceed 0.09% (Table 4), which is acceptable at a substrate concentration close to 100%. Statistical treatment of the results of titration of samples from three laboratory batches of monomecaine (Table 5) showed good reproducibility; the

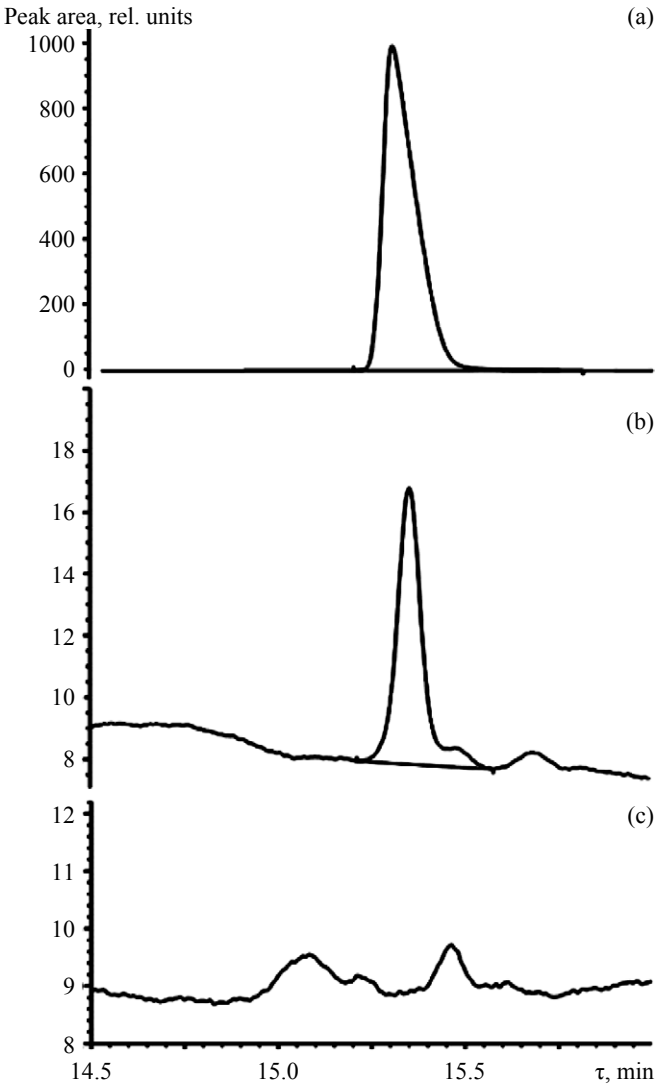


Fig. 2. HPLC chromatograms of solutions of monomecaine (**6**) with a concentration of (a) 214 and (b) 2 μg/mL and (c) blank solution.

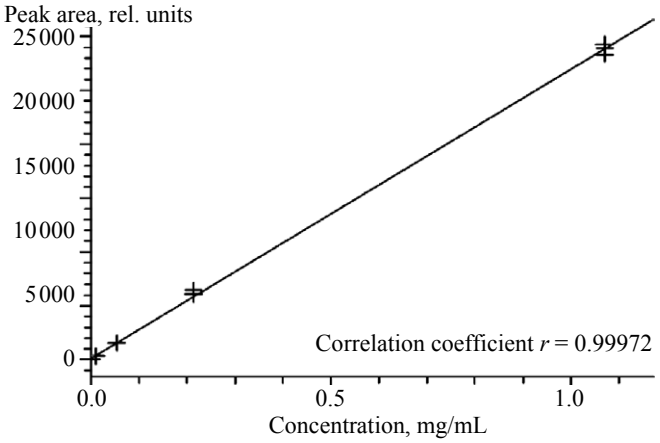


Fig. 3. Calibration curve for HPLC quantification of monomecaine (**6**).

Table 2. Statistical treatment[a] of the results of HPLC quantification of monomecaine (**6**) in the substance (batch no. 250115)

Concentration, mg/mL	$\bar{x}$, mg/mL	S_δ, mg/mL	$S_{\delta av}$, %	$\Delta\bar{x}$, mg/mL
0.80	0.79	0.016	2.00	0.044
1.00	1.01	0.021	2.10	0.059
1.20	1.20	0.023	1.91	0.064

[a] $P = 95\%, f = 4$.

Table 3. Statistical treatment[a] of the results of HPLC quantification of monomecaine (**6**) in three batches

Batch no.	$\bar{x}$, mg/mL	S_δ, mg/mL	$S_{\delta av}$, %	$\Delta\bar{x}$, mg/mL	$\bar{\varepsilon}$, %
250115	1.01	0.0095	0.94	0.026	2.61
170314	1.00	0.0071	0.71	0.020	1.97
050713	0.99	0.0095	0.96	0.026	2.66

[a] $P = 95\%, f = 4$.

Table 4. Statistical treatment[a] of the results of extraction–titration quantification of monomecaine (**6**) in the substance (batch no. 050713)

Concentration level, %	$\bar{x}$, mg/mL	S_δ, mg/mL	$S_{\delta av}$, %	$\Delta\bar{x}$, mg/mL
80	99.46	0.091	0.091	0.084
100	99.28	0.088	0.089	0.082
120	99.36	0.089	0.090	0.082

[a] $P = 95\%, f = 6$.

Table 5. Statistical treatment[a] of the results of extraction–titration quantification of monomecaine (**6**) in three batches

Batch no.	$\bar{x}$, mg/mL	S_δ, mg/mL	$S_{\delta av}$, %	$\Delta\bar{x}$, mg/mL	$\bar{\varepsilon}$, %
250712	99.35	0.068	0.026	0.063	0.17
050713	99.28	0.086	0.033	0.080	0.21
080813	99.35	0.096	0.036	0.089	0.24

[a] $P = 95\%, f = 6$.

concentration of monomecaine in all samples was close to 100%, and the relative error was insignificant.

The extraction–titration method has some advantages over the nonaqueous titration procedure proposed previously. It makes it possible to determine the main component in the presence of decomposition products. It is important here that the molecular weight of the analyte should be no lower than 200. The extraction–titration procedure is promising for use at the stage of development of dosage forms of monomecaine such as infusion solution.

In summary, we have optimized the procedure for the synthesis of 2-(diethylamino)-*N*-(2-methylphenyl) acetamide nitrate, in particular improved the procedure for the preparation of its precursor, 2-chloro-*N*-(2-methylphenyl)acetamide. The latter was obtained with the highest yield in the reaction carried out in anhydrous benzene using 1.5 equiv of chloroacetyl chloride. The purity and structure of the isolated compounds were confirmed by ^{1}H NMR, elemental analyses, and HPLC and TLC data. A procedure for the identification of monomecaine and control of its purity by HPLC has been proposed, and the limit of detection of *o*-toluidine in laboratory samples of monomecaine has been estimated at 0.02%. HPLC and extraction–titration methods have been validated for the quantification of monomecaine. Both methods showed good selectivity, linearity, precision, and reproducibility and can therefore be recommended for the development of a pharmacopeial specification for monomecaine dosage form.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded at room temperature on a Bruker Avance 500 spectrometer (500 MHz, Germany) using DMSO-d_6 as solvent and tetramethylsilane as internal standard. The elemental analyses were obtained on a Perkin Elmer PE 2400 Series II automated CHN analyzer (USA). Reversed-phase HPLC analyses were carried out on an Agilent 1100 chromatograph (USA) equipped with a Kromasil 100-5C18 column (Sweden), 250×4.6 mm, grain size 5 μm; 0.1% aqueous CF_3COOH (A) and acetonitrile (B) were used as eluents at a flow rate of 0.8 mL/min (elution conditions and retention times are given below). Analytical TLC was performed on Sorbfil plates (*Imid* Ltd., Russia); spots were visualized under UV light or by treatment with iodine vapor. Commercially available analytical grade *o*-toluidine, chloroacetyl chloride, and diethylamine were used without further purification.

2-Chloro-*N*-(2-methylphenyl)acetamide (3). A solution of 1 mL (9.32 mmol) of *o*-toluidine in 10 mL of anhydrous benzene (dried over metallic sodium) was cooled to 0°C, and 1.11 mL (14.0 mmol) of chloroacetyl chloride was added. A white solid immediately precipitated. The mixture was evaporated, and the precipitate was washed with water until neutral washings and dried. Yield 1.31 g (76.7%), colorless crystals, mp 111–113°C; published data [13]: mp 102–104°C; R_f 0.57 (benzene–EtOAc, 9 : 1). HPLC: 0–3 min, 100% A; 3–20 min, 0 to 100% B; 20–22 min, 60% B; τ = 20.77 min. ^{1}H NMR spectrum, δ, ppm: 2.20 s (3H, CH_3), 4.30 s (1H, CH_2), 7.10 d (1H, H_{arom}, J = 7.3 Hz), 7.15 d (1H, H_{arom}, J = 7.5 Hz), 7.20 d (1H, H_{arom}, J = 7.4 Hz), 7.40 d (1H, H_{arom}, J = 7.8 Hz), 9.65 s (1H, NH). Found, %: C 58.86; H 5.48; N 7.65; Cl 19.22. $C_9H_{10}ClNO$. Calculated, %: C 58.86; H 5.49; N 7.63; Cl 19.31.

2-(Dimethylamino)-*N*-(2-methylphenyl)acetamide (5). Compound **3**, 9.10 g (49.56 mmol), was dissolved on heating in 110 mL of anhydrous benzene, 12.64 mL (123.9 mmol) of diethylamine was added, and the mixture was refluxed for 5 h. The precipitate of diethylamine hydrochloride was filtered off, the filtrate was evaporated, and the residue was used in the synthesis of **6** without further purification. Yield 9.69 g (88.8%), brown mobile oil, R_f 0.40 (benzene–EtOAc, 9:1). ^{1}H NMR spectrum, δ, ppm: 1.05 d (6H, $CH_2C\mathbf{H}_3$, J = 7.13 Hz), 2.23 s (3H, CH_3), 2.61 d (4H, $C\mathbf{H}_2CH_3$, J = 7.1 Hz), 3.14 s (2H, CH_2), 7.03 d.d (1H, H_{arom}, J = 7.5, 1.1 Hz), 7.18 d.d (1H, H_{arom}, J = 7.6, 1.1 Hz), 7.22 d (1H, H_{arom}, J = 7.4 Hz), 7.90 d (1H, H_{arom}, J = 7.9 Hz), 9.47 s (1H, NH). Found, %: C 70.46; H 9.06; N 13.53. $C_{13}H_{20}N_2O$. Calculated, %: C 70.87; H 9.15; N 12.72.

2-(Dimethylamino)-*N*-(2-methylphenyl)acetamide nitrate (6). A solution of 9.69 g (43.98 mmol) of **5** in 9 mL EtOH was cooled to 0°C, and 2.96 mL (43.98 mmol) of 67% nitric acid was added dropwise. A white crystalline solid separated in 5 min. The mixture was evaporated, and the product was recrystallized from acetone and dried. Yield 9.03 g (78.5%), white crystals, mp 138.5–139.5°C, R_f 0.30 [benzene–Et(*i*-Pr)$_2$N, 3:0.05]. HPLC: 0–3 min, 5% B; 3–20 min, 5 to 60% B; 20–22 min, 60% B; τ = 14.61 min. ^{1}H NMR spectrum, δ, ppm: 1.24 d (6H, $CH_2C\mathbf{H}_3$, J = 7.2 Hz), 2.23 s (3H, CH_3), 3.23 d (4H, $C\mathbf{H}_2CH_3$, J = 6.9 Hz), 4.15 s (2H, CH_2), 7.16 d.d (1H, H_{arom}, J = 7.4, 1.2 Hz), 7.22 d.d (1H, H_{arom}, J = 7.5, 1.2 Hz), 7.27 d (1H, H_{arom}, J = 7.5 Hz), 7.42 d (1H, H_{arom}, J = 7.7 Hz), 9.45 br.s (1H, HNO_3), 9.96 s (1H, NH). Found, %: C 55.23; H 7.73; N 14.70. $C_{13}H_{21}N_3O_4$. Calculated, %: C 55.11; H 7.47; N 14.83.

Quantification of 2-(dimethylamino)-*N*-(2-methylphenyl)acetamide nitrate (6) by HPLC. A 25-mL volumetric flask was charged with 0.025 g of a sample of **6**, 95% of eluent A and 5% of eluent B were added to 2/3 of the total volume, and the mixture was stirred until complete dissolution of **6**. The volume of the mixture was adjusted to 25 mL by adding the same eluent mixture, the mixture was stirred and filtered through a membrane filter (pore diameter 0.45 μm), and the first filtrate portions were discarded. A 20-μL sample of the obtained solution was injected to an HPLC instrument. Elution conditions: 0–3 min, 95% A–5% B; 3 to 20 min, 5 to 60% B; 20–23 min, 40% A–60% B; τ = 14.9–15.4 min (Fig. 2). The concentration of **6** was calculated by the formula

$$X = \frac{S_1 a_0 P}{S_0 a_1},$$

where S_1 is the average peak area of monomecaine in the chromatogram of analyzed solution, S_0 is the average peak area of monomecaine in the chromatogram of standard solution; a_0 is the amount of monomecaine in the standard solution; a_1 is the amount of sample in the analyzed solution; and P is the concentration of monomecaine in the standard sample, %.

Quantification of monomecaine (6) by extraction–titration with sodium lauryl sulfate. A 0.03-g sample of monomecaine substance preliminarily dried

at 100°C until constant weight was dissolved in 10 mL of 0.02 M aqueous HCl in a 100-mL conical flask, 10 mL of chloroform and two drops of a solution of mixed indicator (Butter Yellow–Methylene Blue, 5:1) were added, and the mixture was titrated with a 0.01 M solution of sodium lauryl sulfate under vigorous shaking until the organic phase changed from light yellow to blue. All titrations were carried out in duplicate.

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CONFLICT OF INTERESTS

The authors declare the absence of conflict of interests.

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