

## RESEARCH ARTICLE

# Transferability of the Electrospray Ionization Efficiency Scale between Different Instruments

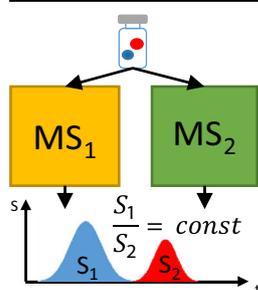
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**Abstract.** For the first time, quantitative electrospray (ESI) ionization efficiencies (IE), expressed as  $\log IE$  values, obtained on different mass-spectrometric setups (four mass analyzers and four ESI sources) are compared for 15 compounds of diverse properties. The general trends of change of IE with molecular structure are the same with all experimental setups. The obtained IE scales could be applied on different setups: there were no statistically significant changes in the order of ionization efficiency and the root mean of squared differences of the  $\log IE$  values of compounds between the scales compiled on different instruments were found to be between 0.21 and 0.55 log units. The results show that orthogonal ESI source geometry gives better differentiating power and additional pneumatic assistance improves it even more. It is also shown that the ionization efficiency values are transferable between different mass-spectrometric setups by three anchoring points and a linear model. The root mean square error of  $\log IE$  prediction ranged from 0.24 to 0.72 depending on the instrument. This work demonstrates for the first time the inter-instrument transferability of quantitative electrospray ionization efficiency data.

**Keywords:** ESI, Ionization efficiency, Different instruments, Mass spectrometry

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## Introduction

Electrospray ionization mass-spectrometry (ESI/MS) is a widely used analytical technique. The mechanisms of ESI are complex and numerous studies [1–15] have been carried out for its elucidation. It has been shown that the electrospray ionization efficiency (IE) [16] of an analyte is influenced by its molecular structure as well as the solvent (including its pH and additives) and the MS system. Despite intense research, the mechanisms of ESI are not fully understood.

For a better understanding of the ESI process, IE scales, in both positive [7, 9] and negative [8] mode, established for fixed solvent compositions are useful. In both modes, IE prediction

models have been obtained [3, 7, 8, 20]. The IE scales and predicting models can help in choosing optimal analysis conditions and enabling semiquantitative analysis of samples without chemical standards (e.g., in metabolomics) [3].

In order to be useful, these scales should be, as far as possible, transferable between different MS setups (i.e., should not depend on the used ionization source designs and mass-spectrometer types). This condition is not easy to fulfill because a number of instrumental factors are known to influence analyte response in ESI-MS, in particular spray voltage, ion transfer capillary temperature, ion transfer capillary voltage, and tube lens voltage [17, 18].

The mass analyzers used most for electrospray ionization mechanism studies are ion-trap [3, 4, 7–10] and triple quadrupole [2, 6, 19, 20]. Only in few cases, direct comparison of the obtained results is possible because in most cases the studied compounds and solvent systems differ. As an example, mutually consistent results were observed by Henriksen et al. [6] and Huffman et al. [19] although they used different ESI source designs (without and with sheath gas), different applied

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voltages (−2.8 kV and −1.5 kV), and different drying gas temperatures (250°C and 350°C). The analytes that were found to be good responders by Henriksen et al. [5] also gave good response in study by Huffman et al. [19]. However, numerical comparisons of ionization efficiency for different instruments cannot be made based on the data published in the literature.

In order to withdraw the influence of the instrument (as well as numerous other influencing factors) a relative measurement approach has been proposed [16], whereby every measurement essentially compares the IE of two compounds simultaneously in the same solution. An extensive IE scale in the positive ion mode has been compiled with this approach [7]. Having two compounds simultaneously in the same solution can lead to asymmetrical mutual suppression of ionization and thereby biased results [16]. In order to avoid this, a different approach—involving separate IE measurements of individual compounds against an external standard compound—has been used for compiling an extensive IE scale in the negative ion mode [8].

Although the scale of reference [7] has been used a lot, there have been no attempts to reproduce even a subset of the measurements on a different MS system with different ion source. The only work known to us, which offers at least some possibility of results comparison is that of Ehrmann et al. [4]. The responses obtained in reference [4] show good consistency with reference [7] except for diphenylamine, which has good response in reference [1] scale but showed weak response in reference [6] study. Both studies used an ion trap mass spectrometer, but different ESI setups (nanoESI [4] and ESI with assisted nebulization [7]), applied voltages (1.5 and 3.5 kV) and temperatures (150 and 350°C) and different solvents (99.5% methanol and 0.5% acetic acid in one case, and 80% (v/v) acetonitrile and 20% of ultra-pure water with 0.1% formic acid in the other studies). Thus, although rather voluminous ESI IE scales exist for negative and positive modes, their validity on different MS systems with different ESI sources has not been demonstrated. Therefore, the validity of prediction models on different instrumental setups has also not been demonstrated.

In this report, we evaluate the dependence of the used ionization source designs and mass-spectrometer types on IE scales. We also evaluate to what extent IE data established on one instrument can be used for predicting IE as well as analyte concentrations on another instrument. Although the first preliminary models to predict ionization efficiency in one solvent are available [3, 7, 8, 20] we use the ionization efficiency scale values to study the transferability to different mass spectrometric setups.

In order to perform meaningful ESI ionization mechanism studies, it is necessary to demonstrate that the same processes are going on in the droplets of the ESI source in different instruments and that these processes can be quantitatively characterized. Therefore, it is very important to perform quantitative comparisons of ionization processes.

In this study, the focus is set on the transferability of ionization efficiencies between different mass spectrometric setups. In order to study the transferability, as many parameters as possible were held constant to study differences caused only

by different instrumentation. The majority of users utilize LC and MS together and, thus, this study is a prerequisite for starting developments in the direction of a standard substance-free quantitation model in LC-MS that considers the effects of analytes, solvent systems, and instrumental parameters.

Here, the used MS setups have four different mass analyzers (ion trap, single quadrupole, triple quadrupole, and ion cyclotron resonance cell) and four different ESI-setups: conventional with 90° geometry, conventional with >90° geometry, Agilent Jetstream, and an in-house developed ion source 3R [21] (both with 90° geometry). Two solvent compositions [80/20 and 20/80 (v/v) of acetonitrile/ultra-pure water with 0.1% formic acid] and 15 analytes [Phe-Phe-Phe-Phe, Ac-Gly-Lys-OMe, 2,6-(NO<sub>2</sub>)<sub>2</sub>-C<sub>6</sub>H<sub>3</sub>-P1(pyrr) phosphazene (see Supplementary Table S1), tetrapropylammonium, tetraethylammonium, triethylamine, 1-naphthylamine, diphenylphthalate, dimethylphthalate, piperidine, pyridine, pyrrolidine, *N,N*-dimethylaniline, cysteine, and glycine] have been studied. For independent validation of the transferability of the ionization efficiency scale between different instruments, mixture of 15 analytes with known concentrations were analyzed on a mass analyzer not included in the comparison study (an FT-ICR-MS), and the analyte concentrations were predicted from ionization efficiency values from reference [7].

## Experimental

### Chemicals

The compounds included in the establishment of the scale were diphenyl phthalate (Riedel-de Haën, Seelze, Germany Assay 99.9% by GC), dimethyl phthalate (Merck, Darmstadt, Germany Assay ≥99% by GC), tetrapropylammonium chloride (Fluka Analytical, Buchs, Switzerland, purum; ≥98.0% (AT)), tetraethylammonium perchlorate (Fluka, Buchs, Switzerland puriss.), *N,N*-dimethylaniline (pure, Reakhim, Moscow, Russia), 1-naphthylamine (pure, Reakhim, Moscow, Russia), piperidine (>99.5%, Sigma-Aldrich, St. Louis, MO, USA), pyrrolidine (>98%, Fluka, Buchs, Switzerland), pyridine (>99.8%, Fluka, Buchs, Switzerland), triethylamine (99%, Aldrich, St Louis, MO, USA), Phe-Phe-Phe-Phe (Bachem, Bubendorf, Switzerland), 2,6-(NO<sub>2</sub>)<sub>2</sub>-C<sub>6</sub>H<sub>3</sub>-P1(pyrr) phosphazene (pure for analysis), Ac-Gly-Lys-OMe (Bachem, Bubendorf, Switzerland), cysteine (>98%, Sigma Aldrich, St Louis, MO, USA), glycine (>99%, Sigma Aldrich, St Louis, MO, USA). The structures and properties are listed in Supplementary Table S1.

For independent validation, the following compounds were additionally analyzed: tetramethylammonium chloride (puriss. p.a., for ion pair chromatography, Fluka Analytical, Buchs, Switzerland), 2-nitroaniline (pure for analysis), benzamide (pure Reakhim, Moscow, Russia), 2,6-dimethylpyridine (pure for analysis), DBU (pure for analysis), acridine (>97%, Fluka, Buchs, Switzerland), tetrabutylammonium perchlorate (≥99.0%, Fluka, Buchs, Switzerland), tetrahexylammonium benzoate (Kodak, Rochester, NY, USA), diphenylguanidine (pure Reakhim, Moscow, Russia)

The used solvents and additives were acetonitrile (J. T. Baker, Deventer, The Netherlands, HPLC grade and Chromasolv Plus for HPLC,  $\geq 99.9\%$  Sigma-Aldrich, St Louis, MO, USA), ultra-pure water (purified with Millipore Advantage A10 MILLIPORE GmbH, Molsheim, France), and formic acid (Fluka, Buchs, Switzerland, 98%) (HCOOH).

### Equipment

The measurements were carried out in the positive ion mode on five different mass-spectrometers with four different ESI sources. The source parameters were held as similar as possible to decrease the variability from instrumental parameters. The first one was an Agilent XCT ion trap mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). For instrument control, Agilent ChemStation for LC rev. A. 10.02 and MSD Trap Control ver. 5.2 were used. Two types of ESI sprayers were used: conventional (see Supplementary Figure S1 in the Supplementary Material) and in-house developed 3-R sprayer [7] (see Supplementary Figure S4). MS and ESI parameters used for the conventional sprayer were: nebulizer gas pressure 15 psi, drying gas flow rate 7 L/min, drying gas temperature 300°C, and only target mass (TM) was optimized (see reference [9]); for 3-R sprayer [21], nebulizer gas pressure 2 psi, drying gas flow rate 10 L/min, drying gas temperature 350°C, and inner capillary gas pressure 12 bar. For both setups the needle voltage was 3500 V.

The second mass spectrometer was a Varian J-320 (Varian Inc., Walnut Creek, CA, USA) triple quadrupole mass spectrometer. For the instrument control, MS Workstation was used. Used ESI source has an angular geometry (see Supplementary Figure S2) and parameters used were: needle voltage 3500 V, drying gas 10 psi, drying gas temperature 300°C, and shield voltage 300 V. The signal was recorded with capillary voltages 30, 40, 50, 60, and 70 V. The highest obtained signal was used.

The third mass spectrometer was a single quadrupole mass spectrometer Single Quad 6100 equipped with the modified [5] Agilent Jet Stream (AJS) ESI Source [22] (Agilent Technologies, Santa Clara, CA, USA; see Supplementary Figure S3). ESI parameters used were: capillary voltage 3500 V, nozzle voltage 600 V, nebulizer gas pressure 15 psi, drying gas flow rate 7 L/min, drying gas temperature 300°C, sheath gas flow rate 1 L/min, and sheath gas temperature 80°C.

The fourth mass spectrometer was a triple quadrupole Agilent 6495 Triple Quadrupole with conventional ESI source and iFunnel ion funnel (Agilent Technologies, Santa Clara, CA, USA; see Supplementary Figure S1). ESI parameters used were: capillary voltage 4000 V, nebulizer gas pressure 14 psi, drying gas flow rate 15 L/min, drying gas temperature 200°C. For instrument control, Agilent MassHunter Workstation Data Acquisition was used.

For independent validation analysis, Varian 910-FT-ICR-MS system was used. Solutions were infused into a mass spectrometer at a flow rate of 0.5 mL/h. For instrument control, MS Workstation and Omega were used. The ESI source has an

angular geometry (see Supplementary Figure S2) and the parameters used were: needle voltage 3500 V, drying gas pressure 10 psi, drying gas temperature 300°C, shield voltage 300 V, and capillary voltage 30 V.

### IE Measurements

Because measuring absolute ionization efficiencies is complicated, we focus on measuring the relative ionization efficiency (RIE, Equation 1) of a compound  $M_1$  relative to  $M_2$ . The RIE measurement method presented in [16] was used. Solutions of  $M_1$  and  $M_2$  were infused and mixed together with the t-piece so that overall solution flow rate into the ESI source was 0.5 mL/h. The ionization efficiencies were measured in two solvent mixtures: (1) 80% (v/v) acetonitrile and 20% of ultra-pure water with 0.1% formic acid; (2) 20% (v/v) of acetonitrile and 80% of ultra-pure water with 0.1% formic acid.

In case of Agilent 6495 Triple Quadrupole, flow injection and calibration graph were used. Ten dilutions of the analyte stock solutions were made (100-, 50-, 33.3-, 25-, 20-, 10-, 5-, 3.33-, 2.5-, and 2-fold) with the solvent by the autosampler and injected to MS. The injection volume was 10  $\mu$ L and the eluent flow rate was 0.2 mL/min. The RIE value is obtained by dividing the slopes of analytical signals [8].

The ionization efficiency scale in reference [7], which lies in the foundation of this work, considers only ionization by protonation. Thus in  $\log RIE$  calculations, the signals of the protonated forms and their fragmentation products are taken into account (the intensities of all those signals were added), but not those corresponding to sodium or ammonium adducts. The fragments are taken into account because it has been proven that they are formed from protonated ions [9]. The consistencies of both ionization efficiency scales—via protonation [7] and via sodium adduct formation [9]—demonstrate that these ionization pathways can be dealt with separately. The responses for adduct formation are dependent on solvent conditions ( $\text{Na}^+$  ions concentration) and, therefore, it is not possible to quantify the sum of protonation and cationization using one single  $\log IE$  value. The ionization efficiency is defined by:  $IE(M_1) = R_i/C_i$  where  $R_i$  is the MS response of the ion  $[M + H]^+$  (plus possible fragments formed from it) at concentration  $C_i$ , as established earlier [16]. The Relative Ionization Efficiency (RIE) of a compound  $M_1$  relative to  $M_2$  is calculated as follows:

$$RIE(M_1, M_2) = \frac{IE(M_1)}{IE(M_2)} = \frac{C_2 R_1}{C_1 R_2} \quad (1)$$

Logarithmic scale was used for better visualization of the results. For one measurement, five different concentration ratios of two analytes were measured and  $\log RIE$  values were averaged.

The IE values were assigned to the compounds on the scales by a two-stage procedure. As we directly measure relative ionization efficiencies of the studied compounds, an anchoring method has to be chosen in order to assign  $\log IE$  values to the compounds. The same anchoring method as in reference [7] was used (based on the  $\log IE$  value of methyl benzoate

arbitrarily assigned to zero). The procedure is explained additionally in Supplementary Scheme S1.

As a first stage, the individual ionization efficiency scales were compiled using the same approach as in reference [23], i.e., the  $\log IE$  values were assigned to the compounds in such a way as to minimize the sum of squared differences  $SS$  between the differences of the directly measured  $\log RIE(M_i, M_j)$  values and the assigned  $\log IE(M_i)$  and  $\log IE(M_j)$  values according to Equation 2. Tetrapropylammonium was used as temporary reference compound ( $M_i$ ) at this stage.

$$SS = \sum_{k=1}^{n_m} \{ \log RIE_k(M_i, M_j) - [\log IE(M_i) - \log IE(M_j)] \}^2 \rightarrow \min \quad (2)$$

The overall number of measurements is denoted by  $n_m$  and  $\log RIE_k(M_i, M_j)$  is the result of  $k$ -th measurement that has been conducted between compounds  $M_i$  and  $M_j$ .

As the second stage, the obtained  $\log RIE$  scales were anchored to literature  $\log IE$  values of the compounds from reference [7] using least squares minimization according to Equation 3. This stage essentially “shifts” the scales obtained with different instruments or ion sources (while keeping their spans and the differences of the  $\log IE$  values within the scales intact) with respect to each other and takes the measurements of all compounds into account, rather than arbitrarily selecting a single reference compound.

The final  $\log IE$  values, that form the characteristic scale on the corresponding mass spectrometer, were obtained by minimizing the squares of  $\log IE$  values differences on different mass spectrometers according to Equation 3:

$$SS_2 = \sum_{i=1}^n [\log IE_1(B_i) - \log IE_2(B_i)]^2 \rightarrow \min \quad (3)$$

where  $\log IE_1(B_i)$  is the  $\log IE$  value of base  $B_i$  obtained by absolute anchoring under the studied conditions on ion trap mass spectrometer and  $\log IE_2(B_i)$  is  $\log IE$  value of base  $B_i$  under the same conditions on a different mass spectrometer.

For anchoring the scales measured with other solvent composition ( $S_i$ ), the MS signal intensities of  $4 \cdot 10^{-6}$  M tetrapropylammonium in both solvent compositions were measured on the same day and the  $\log IE$  value of tetrapropylammonium in a solvent  $S_i$  was calculated using Equation 4:

$$\log IE(S_i) = \log \left( IE(S_1) \cdot \frac{Signal(S_i) \cdot C(S_1)}{C(S_i) \cdot Signal(S_1)} \right) \quad (4)$$

where  $Signal(S_i)$  and  $Signal(S_1)$  are the signal intensities in solvent composition  $i$  and 1 and  $C(S_i)$  and  $C(S_1)$  are the corresponding concentrations of analyte in the respective solvents.

The  $\log IE$  values assigned for each compound in solvent system (2) were obtained by the above mentioned two-stage procedure. For the ion trap scale with the native ion source only the first stage was used. The  $\log IE$  obtained from Equation 4

was used as reference. For the remaining two scales in this solvent system, the second stage was used but the values were anchored to the XCT scale with the native ion source in this solvent system. The measurement uncertainty aspects of this approach have been addressed in reference [24].

The combined standard deviation  $s_c$  of the ionization efficiencies of the compounds on the scales was calculated using Equation 5:

$$s_c = \sqrt{s_{consistency}^2 + s_{anchoring}^2} \quad (5)$$

where  $s_{consistency}$  is the consistency standard deviation of scale and  $s_{anchoring}$  is the arithmetic mean standard deviation of anchoring.

### Independent Validation

For independent validation of ionization efficiency scale inter-instrument transferability, mixture of 15 analytes in acetonitrile/0.1% HCOOH in pure-water 80/20 (v/v) solvent mixture was infused. The pH-independent compounds—tetrahexylammonium, tetraethylammonium and tetramethylammonium—were chosen as calibrants. A correlation line between calibrants  $\log IE$  values [7] and logarithm of sensitivity (signal to concentration ratio) was established. The obtained slope and intercept values were used to predict the concentration  $c_{calc}$  for each analyte according to Equation 6.

$$c_{calc} = \frac{s}{10^{\log IE \cdot slope + intercept}} \quad (6)$$

where  $S$  is the signal amplitude from mass spectra,  $\log IE$  is the tabulated ionization efficiency from reference [7], and slope and intercept are obtained from the correlation line.

## Results

The results of the  $\log IE$  measurements are presented in Tables 1 and 2 and graphically in the electronic supplementary material in Supplementary Figures S5 and S6. Altogether, 145 relative measurements with 10 compounds were carried out during 1 month on every mass-spectrometer setup in two solvent systems. For each studied combination of MS setup and solvent system, an ionization efficiency scale was compiled. The instrument comparison compounds set covers 4.1 orders of magnitude of ionization efficiency values.

Correlation curves between  $\log IE$  values obtained for the different setups are shown in Supplementary Figures S7 to S9. The results of independent validation are presented in Table 4.

## Discussion

From the results in Tables 1, 2, and 3, it can be concluded that ionization efficiency scales obtained in the same solvent system

**Table 1.** The Positive Mode ESI Ionization Efficiency ( $\log IE$ ) Values in Acetonitrile/0.1% HCOOH 80/20 (v/v) on Four Different Mass Spectrometers with Four Different ESI Setups

	Acetonitrile /0.1% HCOOH 80/20 (v/v)				
	XCT <sup>a</sup>	Q <sup>b</sup>	3Q-Varian <sup>c</sup>	XCT-3R <sup>d</sup>	3Q-6495 <sup>e</sup>
Phe-Phe-Phe-Phe	5.23	NA <sup>h</sup>	3.96	5.15	3.68
2,6-(NO <sub>2</sub> ) <sub>2</sub> -C <sub>6</sub> H <sub>3</sub> -P 1(pyrr) phosphazene <sup>f</sup>	5.05	NA <sup>h</sup>	4.34	5.25	4.91
Tetrapropyl ammonium	4.83	5.09	4.53	4.85	4.95
Tetraethyl ammonium	4.13	4.28	4.28	4.05	4.75
Triethylamine	3.98	3.65	3.69	3.45	4.05
1-Naphthylamine	3.84	3.59	3.83	3.58	4.05
<i>N,N</i> -dimethyl aniline	3.68	3.33	3.91	3.49	4.09
Ac-Gly-Lys-OMe	3.67	NA <sup>h</sup>	3.46	3.48	3.90
Diphenyl phthalate	3.60	3.79	3.52	3.13	3.75
Piperidine	3.38	3.52	3.60	2.90	NA <sup>g</sup>
Dimethyl phthalate	3.16	3.52	3.46	3.08	3.14
Pyrrolidine	3.08	3.63	3.44	2.70	NA <sup>g</sup>
Pyridine	2.97	3.10	3.37	2.64	NA <sup>g</sup>
Cysteine	1.88	NA <sup>h</sup>	2.01	1.15	1.78
Glycine	1.36	NA <sup>h</sup>	2.44	1.23	NA <sup>g</sup>
Consistency standard deviation	0.28	0.17	0.31	0.39	0.30
Span	3.87	NA <sup>h</sup>	2.53	4.10	3.18

<sup>a</sup>Agilent XCT ion-trap with orthogonal ESI (see *Equipment* section)<sup>b</sup>Agilent Single Quad 6100 with Agilent JetStream (see *Equipment* section)<sup>c</sup>Varian J-320 (see *Equipment* section)<sup>d</sup>Agilent XCT ion-trap with in-house developed 3R sprayer (see *Equipment* section)<sup>e</sup>Agilent 3Q-6495<sup>f</sup>Phosphazene see structure Supplementary Table S1<sup>g</sup>It is not possible to measure because the lowest  $m/z$  value measurable with this MS system is 100<sup>h</sup>Not measured

on different instruments have, in broad terms, similar IE orders. On all instruments, high responders are phosphazene, tetrapropylammonium, and tetraethylammonium, and low responders are glycine and cysteine. The Phe-Phe-Phe-Phe is quite a high responder for all mass spectrometric setups but especially in the case of ion trap mass analyzer. One reason could be that the ion optics parameters applied to compounds

with higher  $m/z$  value are more efficient than those applied to low  $m/z$  compounds and, therefore, generate extra gain of response. In the middle of the scales there are differences and for some analytes, they are statistically significant. On the other hand, correlation coefficients obtained while comparing data from different instruments range from acceptable to very good ( $R^2$  0.60–0.97) (Table 3). Comparing the obtained  $R^2$  with the combined standard deviations (up to 0.39  $\log IE$  units) and spans (up to 4.1  $\log IE$  units) of the individual scales (see Table 1), the correlations are acceptable. Differences in the ionization efficiency scales for the different setups could be explained by the solution properties, the sprayer properties (i.e., source design) and the mass spectrometer properties (i.e., ion transport and detection).

**Table 2.** The Positive Mode ESI Ionization Efficiency ( $\log IE$ ) Values in Acetonitrile/0.1% HCOOH 20/80 (v/v) on Three Different Mass Spectrometers with Three Different ESI Setups

	Acetonitrile/0.1% HCOOH 20/80 (v/v)		
	XCT <sup>a</sup>	Q <sup>b</sup>	3Q-Varian <sup>c</sup>
Tetrapropyl ammonium	4.38	4.22	3.80
Tetraethyl ammonium	3.74	3.46	3.62
Triethylamine	3.39	3.14	3.56
1-Naphthylamine	3.40	3.22	3.04
<i>N,N</i> -dimethylaniline	3.37	2.97	2.92
Piperidine	2.91	3.00	3.15
Pyrrolidine	2.33	3.01	2.97
Dimethyl phthalate	3.30	3.50	NA <sup>e</sup>
Pyridine	2.41	2.71	2.87
Consistency standard deviation	0.02	0.03	0.03
Combined standard deviation <sup>d</sup>	0.06	0.09	0.12
Span	2.05	1.51	0.93

<sup>a</sup>Agilent XCT ion-trap with orthogonal ESI (see *Equipment* section)<sup>b</sup>Agilent Single Quad 6100 with Agilent JetStream (see *Equipment* section)<sup>c</sup>Varian J-320 (see *Equipment* section)<sup>d</sup>Combined standard deviation calculated using Equation 5<sup>e</sup>Not measured

### Electrospray Source Design and Solution Properties

The scales could differ because of differences in electrospray sources. Indeed, the geometrical ESI source parameters that vary are the dimensions of the needle, the shape of needle tip, the geometry of electrospray setup (e.g., angle between needle and MS inlet capillary, on-axis or off-axis design), and the distance between needle tip and mass spectrometer inlet. Moreover, support gas parameters and voltages, such as the nebulizer gas pressure, drying gas temperature and flow rate, additional gas occurrence, the applied voltage between needle and mass spectrometer inlet, and additional voltage occurrence are different. These source properties are likely to cause

**Table 3.** The Results of Bilateral Comparison *t*-Tests on the Ionization Efficiency Scales Obtained on Different Mass Spectrometers with Different ESI Setups and Linear Analysis Results from Scales Comparison. Slope and Intercept are Obtained so that  $\log IE$  Values from ESI setup 1 are on x axis and  $\log IE$  values, from Setup 2 are on y axis

	Setup 1	Setup 2	Statistical differences	Average difference	Residuals standard deviation	Slope	Intercept	R <sup>2</sup>
80/20	XCT	Q	Pyrrolidine	0.27	0.31	0.85 ± 0.18	0.65 ± 0.67	0.73
	XCT	3Q-Varian	Phe-Phe-Phe-Phe, 2,6-(NO <sub>2</sub> ) <sub>2</sub> -C <sub>6</sub> H <sub>3</sub> -P1(pyr) phosphazene, glycine	0.38	0.29	0.57 ± 0.07*	1.55 ± 0.28*	0.82
	XCT	XCT-3R	-	0.27	0.21	1.12 ± 0.05*	-0.68 ± 0.20*	0.97
	XCT	3Q-6495	Phe-Phe-Phe-Phe, tetraethylammonium	0.33	0.55	0.78 ± 0.18	0.87 ± 0.74	0.66
	Q	3Q-Varian	Tetrapropylammonium, <i>N,N</i> -dimethylaniline	0.23	0.22	0.58 ± 0.13*	1.60 ± 0.49*	0.71
	Q	XCT-3R	Pyrrolidine, pyridine	0.39	0.34	1.05 ± 0.20	-0.54 ± 0.78	0.76
	Q	3Q-6495	<i>N,N</i> -dimethylaniline	0.38	0.42	0.77 ± 0.28	1.11 ± 1.12	0.60
	3Q-Varian	3Q-6495	Tetraethylammonium	0.34	0.28	1.27 ± 0.13	-0.82 ± 0.49	0.91
	3Q-Varian	XCT-3R	Phe-Phe-Phe-Phe, 2,6-(NO <sub>2</sub> ) <sub>2</sub> -C <sub>6</sub> H <sub>3</sub> -P1(pyr) phosphazene, piperidine, glycine	0.57	0.48	1.67 ± 0.20*	-2.65 ± 0.71*	0.85
	XCT-3R	3Q-6495	Phe-Phe-Phe-Phe, tetraethylammonium	0.55	0.53	0.65 ± 0.14*	1.52 ± 0.56*	0.69
20/80	XCT	Q	All except piperidine	0.29	0.24	0.59 ± 0.14*	1.32 ± 0.44*	0.73
	XCT	3Q	all except Tetraethylammonium and triethylamine	0.38	0.24	0.43 ± 0.13*	1.85 ± 0.43*	0.64
	Q	3Q	Tetrapropylammonium, triethylamine	0.20	0.22	0.66 ± 0.18	1.13 ± 0.58	0.69

\*Slope value differs significantly from 1 statistically, and intercept value differs significantly from 0 statistically

differences in electrospray bloom (e.g., in solvent evaporation rate or droplet size variations). This can lead to differences in droplet compositions from where, on average, the ion ejection occurs.

The results show that the ESI sprayer geometry is important. Comparing the spans with *t*-test, there are statistically significant differences only between the scales obtained with the 3Q mass spectrometer and the other instruments (Table 3). The scales obtained with the 3Q mass spectrometer are more than 1.3 times compressed compared with the ones obtained with the other MS systems. One reason could be that in this ESI source, the needle is at approximately 120° with respect to the mass spectrometer inlet capillary as opposed to the orthogonal geometry of the remaining ion sources. Compared with the orthogonal geometry of the remaining ion sources, the analytes have less time to evaporate from the droplets, and most of the droplets are blown to the counter electrode [25]. Voyksner and Lee [26] and Holčapek et al. [27] have shown that the orthogonal ESI source configuration gives better sensitivity than other source designs thanks to the prevention of clogging the MS orifice by nonvolatile materials. Additionally, Tang and Smith [28] and Gomez and Tang [29] have shown that progeny droplets—sources for ions—are ejected in the sidewise direction toward the periphery region of the electrospray. Therefore, orthogonal source designs typically show better sensitivities.

Interestingly, we observed that standard deviation obtained with solvent composition (2) are significantly smaller than with solvent composition (1) in the case of orthogonal pneumatically assisted ESI source and sheath gas assisted Jet Stream. In case of high acetonitrile percentage, the solvent fractionation and drying rate are more affected by the drying and nebulizing gas flow rate and temperature and the addition of a sheath gas

because of the high volatility of the acetonitrile solvent. Electrospray plume obtained with a lower acetonitrile percentage (20%) is less affected by the gas parameters because of the high proportion of less volatile water solvent. The solvent fractionation is less efficient whatever the gas parameters used, which results in similar  $\log IE$  values for the different systems.

Previous studies show that using different electrospray parameters (mainly sheath gas temperature and flow rate as well as capillary voltage) result in different droplet size, droplet composition, and pH [5, 30].

Comparing the ionization efficiencies obtained with the Jet Stream and with the orthogonal pneumatically assisted ESI source, the order of compounds in the scale changes. Additionally, regression analysis shows that the data points do not display a linear relationship. This could be explained by the fact that in the Jet Stream the optimum conditions are very analyte-dependent as shown by Stahnke et al. [31] and Periat et al. [32].

### Mass Spectrometer Properties

In addition to source design, the mass spectrometer may have an effect on ionization efficiency. In previous studies, the ion optics parameters (via optimizing the target mass) of the XCT instrument were optimized and scales with optimized and default ion optics parameters were compared [7, 10]. The optimized ion optics parameters improve the consistency in the scale and between the scales. In this study, we are unable to use the same ion source on different instruments and, therefore, it is not possible to statistically separate the effects of the ion source and the mass spectrometer. As also mentioned in the previous paragraph, the source geometry and the addition of

drying gas affect the desolvation process in ESI. The early stages of ion train devices (i.e., transfer capillary, tube lens) may present different efficiencies with respect to partly desolvated ions.

### Usefulness of Scales

The trends demonstrated by the IE scales obtained on different instruments are the same. Although the  $\log IE$  values as a rule cannot be transferred from one MS setup to another, the scales give general information about ionization efficiency: the statistical analysis of the obtained scales shows that they have differences but they correlate with each other. The order of compounds in the scale does not change remarkably, and the ionization efficiencies are consistent, for the different setups, in the range of half logarithmic unit (equivalent to three times sensitivity difference). The good correlation between the different scales also assures that models built for predicting ionization efficiency are transferable between instruments, and only the coefficients in the model may need some adjustment depending on the instrument.

It can be assumed that this type of adjustment can be easily carried out with measurements of three or more compounds from the scale on the new instrument. According to the obtained intensities, the adjustment of the predictive model can be made. Likely, three anchoring points will be sufficient to scale the ionization efficiencies and use them in semiquantitative analysis as seen below (in “Independent Validation” section).

For assessing the inter-instrument transferability of the ionization efficiency scale, the ionization efficiencies of three compounds—tetrapropylammonium, pyridine, and *N,N*-dimethylaniline—obtained with the ion trap mass spectrometer and with another setup were correlated. In the case of Agilent 6495 Triple Quadrupole, cysteine was used instead of pyridine. The ion trap MS is chosen as reference point for this study because an extensive ionization efficiency scale study for both

positive [7] and negative [8] ionization mode have been compiled with this instrument and the reliability of these scales has been demonstrated [10]. These three analytes were chosen to obtain an interpolative prediction model; tetrapropylammonium is the highest responder, pyridine is the low responder, and *N,N*-dimethylaniline is situated in the middle of the scale measured on the ion trap instrument. In the predictive model, the slopes and intercepts, if statistically significant, were used. The root mean square deviation of the differences between predicted ionization efficiencies on different setups and the measured ionization differences varied from 0.24 (in-house developed 3R [21]) to 0.72 (triple quadrupole Agilent 6495). These are in the same range as the consistency standard deviations of obtained scales. Therefore, we have demonstrated that the built ionization efficiency scale is transferable for different ESI-MS setups and can be used to optimize analysis parameters.

### Independent Validation

The first preliminary models [3, 7, 8, 20] for predicting ionization efficiency are available, but each of them has been set up on a single instrument. In order to demonstrate the inter-instrument transferability the obtained  $\log IE$  values from reference [7] (orthogonal ESI source geometry and ion trap mass analyzer) were applied to predict the concentrations of 12 analytes (seven of them were only used in validation set) on a completely different mass spectrometric setup (approximately 120° ESI source geometry and hybrid mass analyzer that consist of triple quadrupole and FT-ICR). The used validation compound set covers 3.5  $\log IE$  units. The data in Table 4 demonstrate that the concentrations of two compounds (pyridine and tetrapropylammonium) differ 2.0–2.5 times, and for the remaining 10 compounds, the difference is less than two times. The average difference is 1.7 times. This validation gives additional support to the transferability of the  $\log IE$  scale between different ESI-MS setups.

**Table 4.** The Used Concentrations ( $c_{\text{sample}}$ ) and the Predicted Concentrations ( $c_{\text{calc}}$ ) of Independent Validation

	$\log IE^a$	$c_{\text{sample}}$ (mol/L)	$c_{\text{calc}}$ (mol/L)	$c_{\text{calc}}/c_{\text{sample}}$
Tetramethylammonium	2.15	5.25E-06	NA <sup>b</sup>	
2-Nitroaniline	2.44	2.36E-06	3.25E-06	1.38
Benzamide	2.74	9.57E-07	1.42E-06	1.48
Pyridine	2.94	4.89E-06	1.96E-06	0.40
Piperidine	3.16	9.42E-07	4.92E-07	0.52
2,6-Dimethylpyridine	3.41	3.50E-06	4.87E-06	1.39
Triethylamine	3.53	6.66E-07	4.59E-07	0.69
<i>N,N</i> -dimethylaniline	3.72	1.02E-06	5.84E-07	0.57
Tetraethylammonium	3.95	9.40E-07	NA <sup>b</sup>	
Diazabicycloundecene	3.96	8.53E-07	1.57E-06	1.84
Acridine	4.42	9.05E-07	7.95E-07	0.88
Diphenylguanidine	4.61	4.08E-07	2.78E-07	0.68
Tetrapropylammonium	4.97	3.82E-07	1.79E-07	0.47
Tetrabutylammonium	5.13	2.83E-07	1.73E-07	0.61
Tetrahexylammonium	5.65	7.96E-08	NA <sup>b</sup>	

<sup>a</sup>Values from ref. [7]

<sup>b</sup>Values used for calibration

## Conclusion

The main goal of this work was to show that the established electrospray ionization efficiency scale in one solvent was transferable between mass spectrometric setups. Therefore, we compared electrospray ionization efficiency scales obtained on different mass-spectrometric setups for 10 compounds of diverse properties. The general trends of change of ionization efficiency with molecular structure are the same with all experimental setups. However, there are statistically significant differences between quantitative  $\log IE$  values obtained on different setups in the case of many individual compounds, which cannot be related to the structures or properties of compounds in an easy way. The obtained scales can be transferred between MS setups: the root mean square errors of up to 0.72 log units were obtained when transferring a scale from one instrument to another via three anchor components and a linear model.

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