

# Project ILSAP: an inter-laboratory study on accuracy and precision in isotachopheresis

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## ABSTRACT

In a collaborative study by seventeen laboratories, six aqueous sample solutions also containing 0.9% (w/v) NaCl were analysed for lactate and creatinine using isotachopheresis. A split-level study was carried out with three levels of the order of 3, 10 and 30 mmol/l, two sublevels and without blind duplicates. A calibration graph was constructed at five concentrations, using citrate and Tris as internal standards, added on a mass basis. The concentrations in the sample were determined in singular. After elimination of a few outliers using the Grubbs test at a 1% confidence level, data were treated according to ISO 5725. For low, medium and high concentration levels, the repeatability values  $r$  were 0.41, 0.40 and 1.67 mmol/l, respectively, for lactate and 0.63, 0.53 and 1.43 mmol/l, respectively, for creatinine. The reproducibility values  $R$  were 0.59, 1.12 and 2.05 mmol/l, respectively, for lactate and 1.33, 0.88 and 2.66 mmol/l, respectively, for creatinine.

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## INTRODUCTION

This paper describes the results of a collaborative study on capillary isotachopheresis, decided upon by a number of participants during the *7th International Symposium on Capillary Electrophoresis and Isotachopheresis* in the High Tatras, Czechoslovakia, in 1990. The preparation of the collaborative study and analyses were carried out in 1991.

The aim of the study was to evaluate the accuracy and precision of capillary isotachopheresis. It was decided to use universal detection (zone length measurement) because of the simple and sound theoretical basis of this detection principle. The sample components determined should be of interest from a practical point of view and include both cations and

anions. Their number should be limited in order to avoid too much work on calibration. Lactate and creatinine were considered suitable candidates. As for the sample matrix, 0.9% NaCl in an aqueous solution was considered a good compromise between a purely synthetic matrix and a physiological matrix.

After consulting the relevant literature [1], it was decided to organize a split-level study with three levels and two sublevels and without blind duplicates. The levels were chosen such that they were within the dynamic range of the instrument with the same injection volume, depending of course on the sample load of the instrument.

A number of laboratories were invited to take part in the study. Invitations were sent to those laboratories having practical experience with capillary isotachopheresis on an analytical scale for a number of years, resulting in publications and contributions to symposia on isotachopheresis. Eighteen of those agreed to par-

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ticipate. A draft proposal was then sent to the participants, requesting suggestions for improvement. These were incorporated in the final procedure, distributed together with all chemicals and sample solutions.

Results were received from seventeen laboratories. Some of these submitted additional results, obtained under different operating conditions. These results were not averaged. For statistical treatment, a single result was taken from each laboratory. Outlier tests were applied to both calibration data and individual results. For those laboratories having submitted more than one result, these are listed separately. For correlations between individual results and operating conditions, all results, including the sub-laboratory results and the outliers, were taken. The idea behind this was that outliers may have been caused by the operating conditions.

## EXPERIMENTAL

### Equipment

The equipment used was either laboratory made or commercially available, provided that it was designed for analytical and not preparative purposes. The method of injection was either by syringe (in twenty cases, 1–5  $\mu\text{l}$ ) or a fixed volume sample valve (in seven cases, 0.2–30  $\mu\text{l}$ ). The method of detection was universal: a.c. (21), high-frequency (h.f.) (1) or d.c. (5) conductivity detection. The driving current (15–150  $\mu\text{A}$ ) was kept constant during detection and adjusted to the inside diameter (0.2–0.55 mm) and length (133–400 mm) of the separation capillary in order to reach an end voltage acceptable for the proper performance of the instrument. In nine instances column coupling was used.

A strip-chart recorder or a microcomputer was used for registration of the detector signals in such a way that the time resolution (paper speed or A/D sampling rate) was sufficiently high in order to determine zone lengths accurately. The method of zone-length measurement was based on the fact that the zone lengths are marked by an inflection point in the detector signal (a distinct maximum in the time differential of the signal).

### Samples

The sample matrix consisted of 10-ml amounts of 0.9% (w/v) NaCl solution. The sample solutions were prepared in 1-l volumes using an analytical balance with a 0.1-mg digital readout and calibrated volumetric glassware. The sample components to be determined were lactic acid and creatinine, both at 3, 10 and 30 mmol/l concentration levels.

The split-level study included two solutions of high, two of medium and two of low concentration levels of a sample constituent. Sample solutions were labelled A, B, C, D, E and F. An additional practice sample (labelled P) of approximately 6 mmol/l concentration for both constituents in 0.9% (w/v) NaCl was also provided.

All sample solutions were stored in a refrigerator on receipt and analysed as soon as possible.

### Chemicals

Chemicals for leading and terminating operational systems and for calibration were provided by the organizers in sufficient amount and of the highest purity normally available. Care was taken that the chemicals were from a single production batch. These chemicals were a generous gift from Merck (Darmstadt, Germany).

All solid chemicals were stored in a dry location at room temperature. In addition, lithium lactate was first dried in an oven for 3 h at 110°C and subsequently stored in a desiccator (a 1% mass loss was observed).

### Operational systems

All operational systems were made up in at least 1-l amounts, dissolved in deionized water and stored in a refrigerator for not longer than 2 weeks.

The operational system for anionic analysis was as follows:

leading electrolyte:	0.01 mmol/l histidine
	0.01 mol/l histidine hydrochloride
terminating electrolyte:	0.005 mol/l glutamic acid
	0.01 mol/l histidine

and that for cationic analysis was as follows:

leading electrolyte:	0.01 mol/l sodium glutamate
	0.002 mol/l glutamic acid
terminating electrolyte:	0.005 mol/l glutamic acid.

Prior to the analysis of standards or samples, a blank run was performed using the same current and recording paper speed as in the subsequent analyses. No detectable step height should have occurred at the step height of either sample component or internal standard.

#### *Calibration and analysis*

Determinations of standards and samples were carried out using an internal standard (I.S.). For cationic analyses tris(hydroxymethyl)aminomethane (Tris) was used and for anionic analyses citric acid. Each time, 1–5 ml of I.S. solution were added to an equal volume of sample/standard solution using a calibrated pipette. These dilutions were carried out on a mass basis using an analytical balance with a 0.1-mg readout.

The concentration of the I.S. solution was 10.00 mmol/l for both analytes. The anionic and cationic internal standards were combined in one solution, in deionized water.

Five standard solutions were prepared, having sample component concentrations of 0, 2.00, 5.00, 15.0 and 30.0 mmol/l. Again, standard solutions for anionic and cationic analyses were combined in one solution, also containing 0.9% (w/v) NaCl. The combinations were such that 0 mmol/l creatinine was combined with 30.0 mmol/l lithium lactate, 2.00 mmol/l creatinine with 15.0 mmol/l lithium lactate, etc. Participants were advised to prepare 1-l volumes of each.

#### *Injection and injection sequence*

For each analysis, equal amounts of I.S. and sample/standard were mixed as described previously and injected. The amount injected was adjusted to the separation capacity of the instrument. With equipment using syringe injection, a fixed-volume adapter was used. When using sample valve injection, a dilution could be necessary. Deionized water was used in that event.

For each of the two sample components to be determined (creatinine and lactic acid), all injections took place in 1 day. This meant a minimum of twelve injections per day. If any of the injections or the blank run was “unsuccessful”, it could be repeated, but the sequence of injection remained unchanged. If, as a result, a series of injections could not be completed in 1 day, the sequence was started from the beginning. The sequence of injection was such that standard and sample solutions were alternately injected in approximately increasing concentration.

#### *Submission of results*

Results were submitted on a computer diskette, containing a program to gather raw data and calculate results according to the procedure described above. Laboratory data were stored in a binary file with a file name extension LAC or CRE. Isotachopherograms of a blank, a standard and a sample run were also submitted.

When submitting sub-laboratory results in addition (different equipment and/or operator), an additional digit was added to the laboratory code.

#### TREATMENT OF EXPERIMENTAL DATA

##### *Calculations carried out by all participants*

For each of the analyses, step heights of standard/sample components were measured relative to that of the I.S. and used for within-laboratory identification purposes. They were not reported. For each of the analyses, zone lengths of standard/sample components were measured relative to that of the I.S.:

$$RZL = \text{zone length}_{\text{standard/sample}} / \text{zone length}_{\text{I.S.}} \quad (1)$$

The 1:1 dilution of standard/sample and internal standard solution was carried out on a mass basis. The relative corrected zone length (RCZL) was then calculated as

$$RCZL = 1.0058RZL \cdot \text{mass}_{\text{I.S.}} / \text{mass}_{\text{standard/sample}} \quad (2)$$

In this equation, the factor 1.0058 is the ratio of the average density of the sample and the average density of the internal standard solution. A calibration graph of the five standard solutions was then constructed using linear regression [2]. The slope and intercept of the calibration graph of relative corrected zone length (*RCZL*) vs. concentration (*c*) were finally reported:

$$RCZL_{\text{standard}} = \text{slope} \cdot c_{\text{standard}} + \text{intercept} \quad (3)$$

The relative corrected zone lengths, *RCZL*, as calculated with eqn. 2, were now considered *y*-data and the concentrations in the calibration solutions *x*-data. The slope, intercept and correlation coefficient were then calculated in the usual manner [2].

The sample concentrations were then calculated from

$$c_{\text{sample}} = (y_c - \text{intercept})/\text{slope} \quad (4)$$

where  $y_c$  is the relative corrected zone length (*RCZL*) of the sample.

The calculation of the following parameters might require some explanation. First the following quantities, also needed for the correlation coefficient, were determined:

$$S_{xx} = \sum_{i=1}^N (x_i - x_{\text{mean}})^2 \quad (5)$$

$$S_{yy} = \sum_{i=1}^N (y_i - y_{\text{mean}})^2 \quad (6)$$

where  $x_{\text{mean}}$  and  $y_{\text{mean}}$  are mean values of standard concentrations and corresponding *RCZL*, respectively, and *N* is the number of calibration points (*N* = 5). The standard deviation about regression,  $s_{\text{reg}}$ , and the standard deviation of the slope,  $s_{\text{slope}}$ , for the particular laboratory were then calculated:

$$s_{\text{reg}} = [(S_{yy} - \text{slope}^2 \cdot S_{xx})/(N - 2)]^{1/2} \quad (7)$$

$$s_{\text{slope}} = (s_{\text{reg}}^2/S_{xx})^{1/2} \quad (8)$$

Finally, the standard deviation,  $s_c$ , of the results obtained from the calibration graph was calculated:

$$s_c = \frac{s_{\text{reg}}}{\text{slope}} [1/M + 1/N + (y_c - y_{\text{mean}})^2 / S_{xx} \cdot \text{slope}^2]^{1/2} \quad (9)$$

Here *M* = 1 because results were obtained from a measurement performed in singular. This value  $s_c$  was used as the within-laboratory standard deviation.

#### Elimination of outliers [3]

After receiving all results from all participants, data were combined into one database in which first outliers were determined. The outliers distinguished were laboratory outliers and individual results outliers.

First, laboratory outliers were detected by observing the calibration data, for instance if the calibration graph parameters slope, intercept or correlation coefficient deviated substantially from the laboratory-to-laboratory average. We applied the Grubbs outlier test [3] for a 1% confidence level for any of the three parameters, using the laboratory-to-laboratory standard deviation of these values, with the restriction that for the correlation coefficient, which may not be normally distributed, only outliers on the lower side were considered.

After elimination of the laboratory outliers, individual results outliers were detected by comparing the individual value with the mean. Here we also applied the Grubbs outlier test for a 1% confidence level using the laboratory-to-laboratory standard deviation. The Grubbs test was applied to check if the highest or lowest value was an outlier. If this was the case, the outlier was removed and the mean and standard deviation were calculated again. This was repeated until there were no more outliers. Outliers are indicted by asterisks in the tables.

#### Repeatability and reproducibility [4]

In the split-level study, each of the sub-levels was analysed once. For each of the three concentration levels, the following quantities were then calculated according to the required procedure [4]: the repeatability standard deviation  $s_r$  and variance  $s_r^2$ , the between-laboratory standard deviation  $s_L$  and variance  $s_L^2$ , the reproducibility

standard deviation  $s_R$  and variance  $s_R^2$  and the grand mean  $m$ . The repeatability values  $r$  and the reproducibility values  $R$  are 2.8 times  $s_r$  and  $s_R$ , respectively.

## RESULTS AND DISCUSSION

### Calibration graph data

First, the statistics of the calibration graphs were determined, using the mass-corrected internal standard method described. Here, one laboratory outlier (laboratory I) had to be eliminated first because of a correlation coefficient for lactate that was obviously too low (0.9955 compared with a minimum value of 0.9990 for the others).

The lactate calibration graphs had an average slope of 0.04807 with a standard deviation of 0.00325, an average intercept of 0.00676 with a standard deviation of 0.0123 and an average correlation coefficient of 0.99980.

The creatinine calibration graphs had an average slope of 0.10166 with a standard deviation of 0.00813, an average intercept of  $-0.00769$  with a standard deviation of 0.0154 and an average correlation coefficient of 0.99977.

The relative standard deviations of the calibration graph slopes (6.8 and 8.0% for lactate and creatinine, respectively) indicate that the method can be transferred and used without recalibration in different laboratories if the required precision is 10%, a value not uncommon in routine analysis.

### Individual results

The results of the study are reported in two different ways. For the collaborative study, results of different laboratories were compared. Therefore, from each laboratory one result was taken and sub-laboratory results were disregarded. Then, the results were checked for outliers, according to the criteria mentioned, first for laboratory outliers, then for individual outliers. The data remaining were used to calculate averages, standard deviations and other parameters according to ISO 5725 [4].

Results of lactate and creatinine determinations are summarized in Tables I and II, in which also the standard deviation of the concentration

of an average sample ( $C$ ) is given. This parameter  $s_c$  is a more sensitive parameter for the quality of the regression than the correlation coefficient. Considerable differences between laboratories are shown. The outlier status of laboratory I for lactate is obvious. After elimination of the corresponding value, the average within-laboratory standard deviations for lactate and creatinine are approximately the same (0.2 mmol/l for lactate and 0.3 mmol/l for creatinine). The  $s_c$  value is directly related to the detection limit of the procedure used. Note that significant differences between the laboratories exist: 0.02–0.7 mmol/l for lactate and 0.03–0.6 mmol/l for creatinine. This is related to some extent to the relative sharpness of the zone transitions, as can be seen in Fig. 1, an example of typical lactate isotachopherograms.

Owing to different non-linearities of the detector electronics, significant laboratory-to-laboratory differences in relative step height (*RSH*) values for lactate remain. The within-laboratory standard deviation of the *RSH* values, however, is generally much better (1% or less). Finally, the citrate step is not perfect in several instances, an effect we have observed before when analysing multivalent ions with a.c. conductivity detection.

Mixed zones were reported by some laboratories determining creatinine with a non-corrected version of the procedure, where the pH was too low owing to a glutamate counter-ion concentration that was too high. An erratum was sent to correct this. Laboratory J also reported a mixed zone between leading zone and citrate in the lactate determination. The sample was diluted prior to injection to avoid this effect. The *RSH* value of this mixed zone is low, so that the step from leading zone to mixed zone is easily overlooked and mistaken for the leading zone. For instance, laboratory A, using equipment identical with that of laboratory J, did not report such a mixed zone.

### Repeatability and reproducibility

Calculations according to ISO 5725 [4] were carried out with each of the three levels of both lactate and creatinine. The results are given in Table III.

TABLE I  
LACTATE RESULTS IN mmol/l

Laboratory	Sample <sup>a</sup>						$s_c^b$
	A	B	C	D	E	F	
A	3.18	4.48	11.21	10.33	28.02	31.19*	0.28
B	3.04	4.16	10.17	9.16	26.87	28.55	0.15
C	2.96	4.15	9.92	8.67	28.43	27.24	0.48
D	3.11	4.15	10.30	9.07	27.61	28.19	0.02
E	3.30	4.32	10.66	9.49	27.52	28.14	0.38
F	3.07	4.09	10.16	9.33	27.02	28.52	0.06
G	2.98	4.48	11.23	9.75	28.13	29.07	0.24
H	nd <sup>c</sup>	4.05	10.16	9.24	26.96	27.82	0.04
I	6.21*	3.90*	7.91*	6.60*	18.99*	18.67*	1.50*
J	3.23	4.26	10.20	9.32	26.73	27.59	0.18
K	2.51	3.89	10.01	9.16	27.04	28.75	0.29
L	3.14	4.15	10.22	9.14	26.72	27.89	0.06
M	3.13	4.19	10.40	9.26	27.58	28.79	0.13
N	2.66	4.29	10.56	9.85	26.17	28.83	0.67
O	3.16	4.11	10.32	9.20	26.58	27.81	0.07
P	3.11	4.25	10.22	9.15	26.43	27.78	0.14
Q	3.13	4.06	9.93	9.14	26.62	nd	0.28
<i>n</i>	15	16	16	16	16	14	16
True	3.04	4.07	10.10	9.04	26.98	28.15	
Mean	3.05	4.19	10.35	9.33	27.15	28.21	0.22
S.D.	0.21	0.15	0.39	0.38	0.66	0.55	0.18

<sup>a</sup> Outliers are marked with asterisks.

<sup>b</sup>  $s_c$  is the within-laboratory standard deviation of the concentration of sample, C.

<sup>c</sup> nd = Not determined.

In terms of repeatability standard deviation, both low and medium concentration levels are around 0.2 mmol/l whereas the 27 mmol/l concentration level scores significantly higher: 0.6 mmol/l for lactate and 0.5 mmol/l for creatinine. A relative value of 1–2% over the whole concentration range, however, is acceptable.

Concerning the between-laboratory standard deviation, a high value for the 27 mmol/l creatinine level is observed. Here the true and mean values also differ significantly. A possible reason might be that for this level, decomposition in some laboratories cannot be excluded, in spite of the precautions taken. Some delivery problems reported might also be at the origin.

#### Sub-laboratory results

A considerable number of participants also reported results from sub-laboratories with dif-

ferent equipment, operating conditions, operator or data evaluation method. It was considered incorrect to average these results together with data from other laboratories. The experimental data supplied describe the experimental conditions in considerable detail. An additional aim of this study was to correlate results with experimental data. For these correlations, all results submitted were used, including the outliers. The reason is that outliers are possibly outliers because of experimental conditions. In this way outliers and other results may be explained.

When comparing lactate sub-levels for all (sub-)laboratories, it was observed that in only one instance were the lower and higher sub-levels distinguished incorrectly. When doing the same with the creatinine sub-levels, it was observed that in only two instances were the lower and higher sub-levels distinguished incorrectly. In only a few instances did the difference be-

TABLE II  
CREATININE RESULTS IN mmol/l

Laboratory	Sample <sup>a</sup>						$s_c$ <sup>b</sup>
	A	B	C	D	E	F	
A	28.46	26.81	8.37	9.68	3.18	4.21	0.61
B	27.13	26.17	8.27	9.51	2.44	3.10	0.24
C	27.63	27.86	8.12	9.75	2.39	2.68	0.48
D	27.79	27.09	8.72	9.80	3.27	4.33	0.46
E	28.28	26.28	8.94	10.12	2.85	3.66	0.19
F	27.23	25.96	8.69	9.93	2.84	3.69	0.03
G	26.71	24.70	8.34	9.39	2.69	3.33	0.41
H	nd <sup>c</sup>	25.77	7.95	9.82	2.73	2.98	0.31
K	27.54	24.91	7.59	9.45	2.37	2.32	0.35
L	27.55	26.90	8.50	9.79	2.98	3.76	0.19
M	28.07	26.47	8.36	9.69	2.94	3.74	0.12
N	25.54	24.37	6.92	8.50*	1.47*	1.98	0.09
O	27.37	26.05	8.55	9.79	2.79	3.39	0.14
P	27.25	25.79	8.39	9.60	2.66	3.52	0.20
<i>n</i>	13	14	14	13	13	14	14
True	28.24	27.05	8.94	10.30	3.03	3.86	
Mean	27.43	26.08	8.26	9.72	2.78	3.34	0.27
S.D.	0.74	0.96	0.51	0.20	0.28	0.67	0.17

<sup>a-c</sup> See Table I.

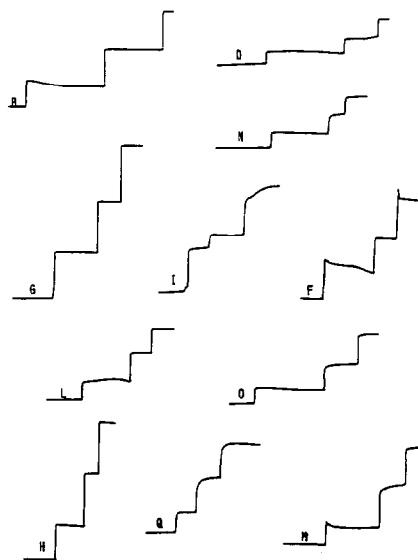


Fig. 1. Isotachopherograms for a typical lactate analysis. Time is from left to right; the vertical axis shows increasing resistance. Zones are chloride (leading), citrate (internal standard), lactate (sample) and glutamate (terminator). The time base is not the same; the figure intends to illustrate qualitative differences.

tween two sub-laboratory results significantly exceed the repeatability standard deviation,  $s_r$ , of this laboratory.

### Three calibration methods

For two injection techniques, syringe and loop, three different calibration methods were compared: (i) I.S. + M: internal standard on a mass basis, using the dimensionless relative corrected zone length  $RCZL$ , as  $y$ -data in the calibration graph; (ii) I.S.: internal standard, using the dimensionless relative zone length,  $RZL$ , as  $y$ -data in the calibration graph; and (iii) E.S.: external standard, using the absolute zone length,  $ZL$ , in arbitrary units as  $y$ -data for the calibration graph.

In order to compare the three methods, the relative standard deviations of the slope of the calibration line ( $s_{\text{slope}}/\text{slope} \cdot 100\%$ ) were calculated. As with the  $s_c$  values, significant differences between laboratories were observed. Comparing average values therefore does not lead to general conclusions. In some instances, using an internal standard significantly improved the qual-

TABLE III  
REPEATABILITY AND REPRODUCIBILITY IN mmol/l (ISO 5725 [4])

Parameter	Lactate			Creatinine		
	Low	Medium	High	Low	Medium	High
Number	15	16	14	13	13	13
True mean value	3.56	9.57	27.57	3.45	9.60	27.65
Grand mean $m$	3.62	9.84	27.67	3.11	9.04	26.77
Repeatability S.D. $s_r$	0.15	0.14	0.60	0.23	0.19	0.51
Between-laboratory S.D. $s_L$	0.15	0.37	0.43	0.42	0.25	0.80
Reproducibility S.D. $s_R$	0.21	0.40	0.73	0.47	0.31	0.95
Repeatability value $r$	0.41	0.40	1.67	0.63	0.53	1.43
Reproducibility value $R$	0.59	1.12	2.05	1.33	0.88	2.66

ity of calibration. In others, where I.S. calibration significantly decreased the performance, it must be concluded that the I.S. method was not properly carried out.

In cases where mass correction did not improve I.S. calibration, it must be concluded that the 1:1 dilution on a volume basis was carried out with sufficient precision, in other words, mass correction did not improve the precision.

#### *Injection and reproducibility*

The possible difference between syringe and loop injection can be investigated by averaging the standard deviation of the regression,  $s_{reg}$ , or the within-laboratory standard deviation,  $s_c$ , of a sample of medium concentration  $C$  (see Table IV).

From the results for both lactate and creatinine it can be concluded that loop injection

gives a slightly better repeatability than syringe injection. The difference is not very distinct statistically. In addition, it was seen that in spite of using syringe injection, some laboratories performed excellently, explaining the large standard deviation  $s_c$  for syringe injection.

#### *Reproducibility of I.S. zone length*

As the concentration of I.S. is in principle the same in all samples and calibration solutions, the relative standard deviation of the I.S. zone length within a laboratory can be attributed mainly to differences in injection volume. Significant differences between individual laboratories were observed (1-35%). Overall, it cannot be concluded that loop injection performs better in this respect.

#### *Accuracy*

From the bottom lines of Tables I and II, conclusions can be drawn regarding the accuracy of the procedure for lactate and creatinine, respectively. It appears that in most instances the true and mean values differ less than the between-laboratory standard deviation. This is not so, however, with some of the creatinine samples, where the mean is always less. In spite of the provisions taken in the procedure, decomposition of the sample cannot be excluded.

Accuracy problems may be encountered in individual laboratories due to overloading. This cannot be analysed from the data provided because an unknown number of laboratories used column coupling without explicitly stating

TABLE IV  
INJECTION AND REGRESSION

Injection	Lactate		Creatinine	
	$s_{reg}^a$	$s_c^a$	$s_{reg}^a$	$s_c^a$
Syringe	$n = 18$	$n = 18$	$n = 15$	$n = 15$
Mean	0.017	0.347	0.036	0.355
S.D.	0.024	0.408	0.054	0.462
Loop	$n = 7$	$n = 7$	$n = 6$	$n = 6$
Mean	0.005	0.124	0.016	0.174
S.D.	0.003	0.080	0.005	0.047



so. In these instances, the effective volume of the capillary between injection and detection cannot be calculated from the inside diameter and length. In addition, those laboratories using loop injection reported the injected volume and not the dilution factor, so that sample load calculations are impossible.

Laboratory J reported problems with the occurrence of mixed zones. The sample was diluted with water to prevent mixed zone formation. How would mixed zones affect the results? Consider lactate, where the citrate internal standard migrates directly behind the leading ion. A mixed zone between leading zone and citrate, if not noticed, may lead to a systematically lower citrate zone length and a different calibration graph slope. As the citrate concentration in all injections is the same, a very reproducible injection may lead to a mixed zone length that is almost constant. Otherwise, a larger standard deviation is expected, in addition to a different slope.

#### CONCLUSIONS

Overall, the procedure using an internal standard yielded good results. A weighing correction of the 1:1 dilution step probably did not add to the precision of the method. The linearity over the concentration range used was excellent. The standard deviation of a single determination, using the calibration graph, however, showed large differences between the individual laboratories. In general, only few outliers were reported. It can be concluded that isotachopheresis is a technique suitable for accurate and precise determinations using a straightforward procedure that is easily transferred from one laboratory to another.

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