Enzytec™ *Liquid* D-/L-Lactic acid – fully-automated application

Art. No. E8240

Verification report

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1. Automation on a Pictus 500 device

1.1 General

The re-usable cuvettes in the Pictus device have a maximal volume of 650 µL with a light path of 0.6 cm which makes a calibration necessary. Therefore, reagent volumes of the manual application are divided by 10. To give a user the maximum flexibility, three different applications with different measurement ranges depending on the sample volume of 2, 10, and 100 µL are provided. Volumes of reagent 1 and reagent 2 were reduced to 200 µL and 50 µL, respectively and are not changed for the three different applications. The Pictus 500 can automatically rerun samples and change between these three applications in case the concentration is below or above the measurement range.

1.2 Calibrators

High range application: four point polynomial calibration with 0 mg/L (water), 250 mg/L, 1000 mg/L and 2500 mg/L (R-Biopharm, E8465, contains 5001 mg/L of L-lactic acid and 5000 mg/L D-lactic acid)

Basic range application: four point polynomial calibration with 0 mg/L (water), 50 mg/L, 200 mg/L and 500 mg/L (use E8465 and dilute with water)

Sensitive range application: four point polynomial calibration with 0 mg/L, 5 mg/L, 15 mg/L, and 50 mg/L (use E8465 and dilute with water)

1.3 Analysis

- Add 200 µL reagent 1
- Add sample, control or calibrator/water; volumes depend on the application:
    a. 2 µL for High range application
    b. 10 µL for Basic range application
    c. 100 µL for Sensitive range application
- It is recommended to pipette n=4 replicates for each calibrator
- Incubate for 2 min at 37 °C (99 °F)
- Read A1 at 340 nm
- Add 50 µL reagent 2
- Incubate for 10 min at 37 °C (99 °F)
- Read A2 at 340 nm

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1.4 Calculations

The Pictus 500 device will calculate a linear calibration function from the single calibrators and use this function to calculate concentrations for unknown samples and control solutions.

1.5 Recommendations for other automates

The ratio of 4:1 for reagent 1 and 2 should not be changed and the sample volume should not be bigger than twice the volume of reagent 2. A calibration must be performed but is often stable for several days so that it must not be repeated on every day. Control solution(s) should be analyzed with every run to check the validity of the calibration. In case these control solutions are not within specifications, a re-calibration must be done.

2. Sample preparation

The sample preparation for the automated test method is identical to the sample preparation for manual testing.

3. Criteria for acceptance

Recovery of aqueous standard solutions on automated devices is identical compared to the manual method and should be within 100 ± 5 %.

4. Automation on a Pictus 500 spectrophotometric analyzer

4.1 Comments on validation parameters independent on automation

Side-reactivity to other related organic acids or other substances and interfering substances will not be characterized on the automated analyzer because there is no know effect that an automated process will change the reactivities towards these substances.

The pipetting environment within a closed automate is much more regulated than the normal laboratory environment (including the analyst). For measurement, all reagents are cooled at 8 ± 2 °C (42 - 50 °F) while the reaction zone where the analysis takes place, is tempered to 37 °C (99 °F). This ensures a quick enzymatic reaction and highly reproducible results.

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Therefore, the characterization of incubation times and temperatures was not repeated. Incubation at 37 °C (99 °F) and the necessary incubation times are described for the 4 mL cuvette manual application (see validation report).

There is also no practical reason to analyze test kit components that were tested for their stabilities against transport, freezing, and short-term storage at 37 °C.

The Pictus 500 can automatically change between the three applications in case the concentration is below or above the Basic measurement range. Therefore, it was decided not to characterize each application for an LoD.

4.2 LoQ (Limit of Quantification)

The lower end of the measurement range is the limit of quantification where acceptable recovery and precision are met. Our internal requirements are a recovery higher than 95 % and an RSD equal or lower than 10 %. For each of the three applications, aqueous solutions with different concentrations of D-/L-Lactic acid were analyzed at least five times. The concentration was calculated from the calibration of the system.

Figure 1 shows the results for the Basic range application with a sample volume of 10 µL which is – despite a factor of 10 in volumes – the identical ratio of sample volume to reagents as the manual format with a sample volume of 100 µL, 2000 µL reagent 1 and 500 µL reagent 2. The automated analyzer has an LoQ of 7.5 mg/L using the criteria described before. The manual format exerted a calculated LoQ of about 5 mg/L but the precision at this point is still not sufficient. Data from a precision plot gave the same LoQ of 7.5 mg/L for the manual format.

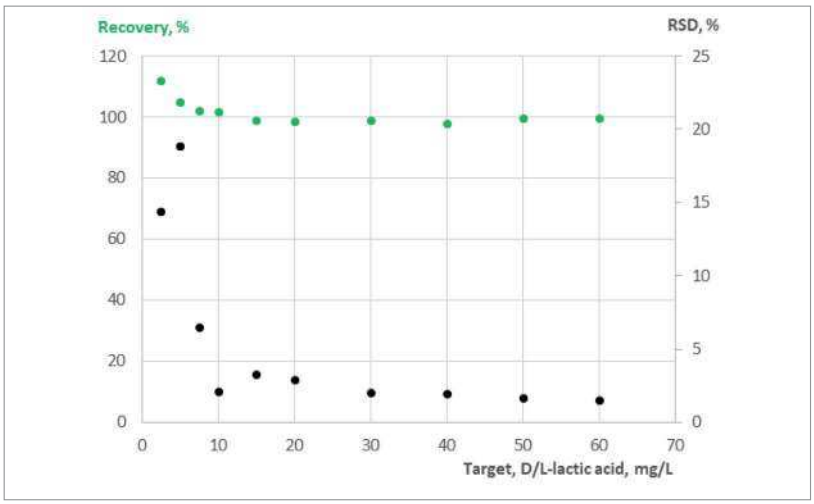

Figure 1: Confirmation of LoQ for the Basic range application with 10 µL sample volume.

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Since both lactic acids are often present at quite high concentrations in food such as fermented milk products, wine, and fermented vegetable (juices), the automated High range application with a low sample volume of 2 µL was introduced to analyze these matrices without dilution prior to measurement. As can be seen in figure 2, the LoQ for this application is 37.5 mg/L. This application was not investigated for the manual format because this would require sample volumes of 20 µL which is challenging for untrained analyst. The Pictus 500 shows RSD values at or below 5 % for a sample volume of 2 µL.

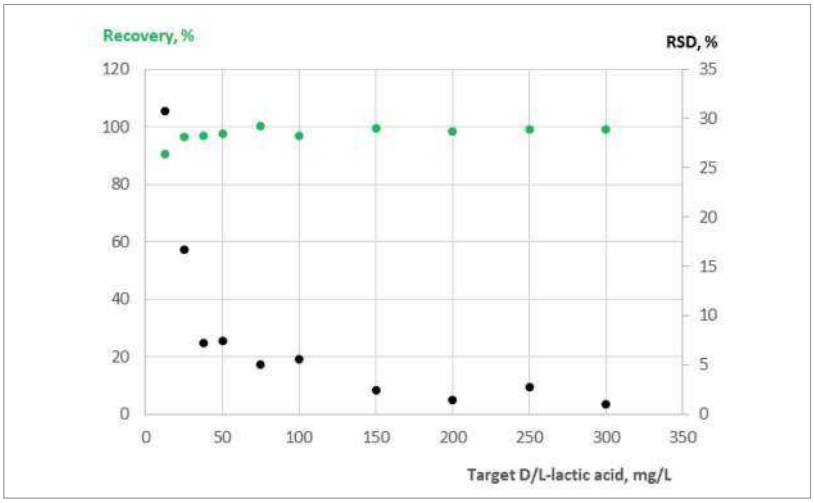

Figure 2: Confirmation of LoQ for the High range application with 2 µL sample volume.

In case trace analysis of lactic acid is necessary, the Sensitive range application with a sample volume of 100 µL was investigated for its LoQ (fig. 3). An LoQ of 4 mg/L can be claimed for this application where recovery and precision requirements were met. If a lower recovery of 90 % is accepted for LoQ the value would be 1.5 mg/L.

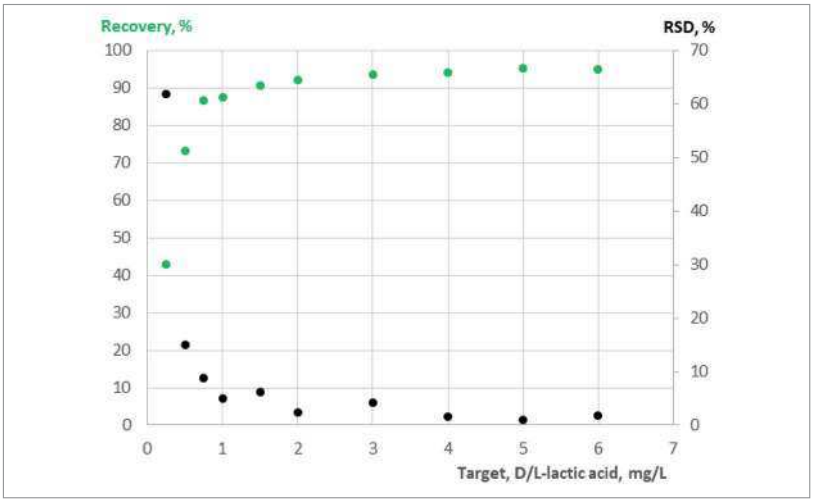

Figure 3: Confirmation of LoQ for the Sensitive range application with 100 µL sample volume.

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4.3 Linearity

The most important parameter for an automated application is the linear range because in case of enzymatic analysis the analyte is often always present in the sample and its proper quantification only depends on the proper choice of sample volume and calibration. For each of the three applications the optimal linear measurement range was characterized. Figure 4 shows that the upper measurement range is 625 mg/L for a sample volume of 10 µL.

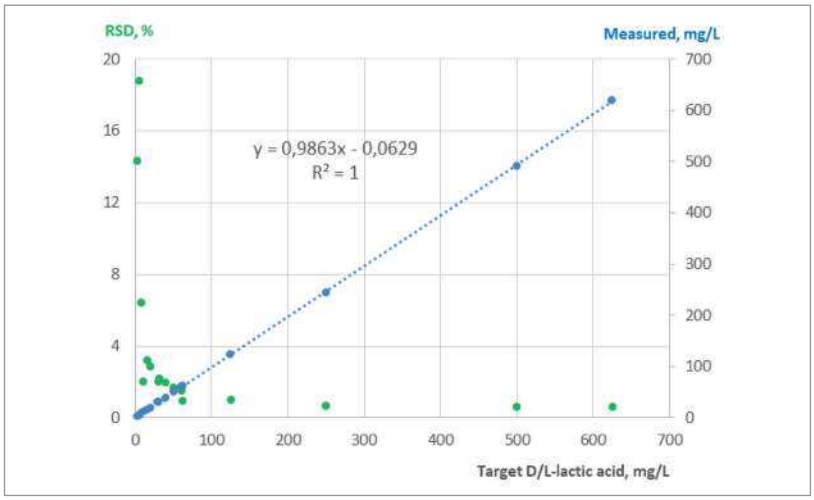

Figure 4: Characterization of linearity for the Basic range application with 10 µL sample volume.

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For the High range application with a sample volume of 2 µL, the upper measurement range is 3125 mg/L, see figure 5. This is a factor of five compared to the Basic range application and perfectly fits to the increased sample volume of 10 µL.

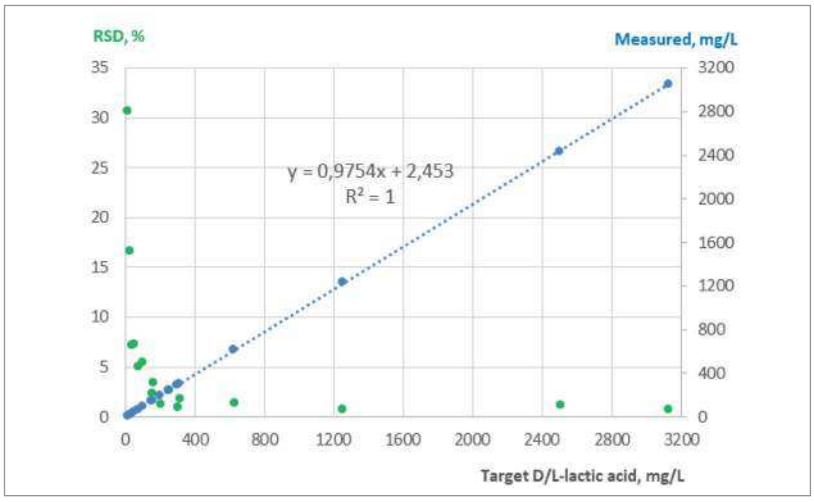

Figure 5: Characterization of linearity for the High range application with 2 µL sample volume.

For the Sensitive range application with a sample volume of 100 µL the upper measurement range is 62.5 mg/L (fig. 6). It is always recommended to include control samples at the upper range to check for linearity.

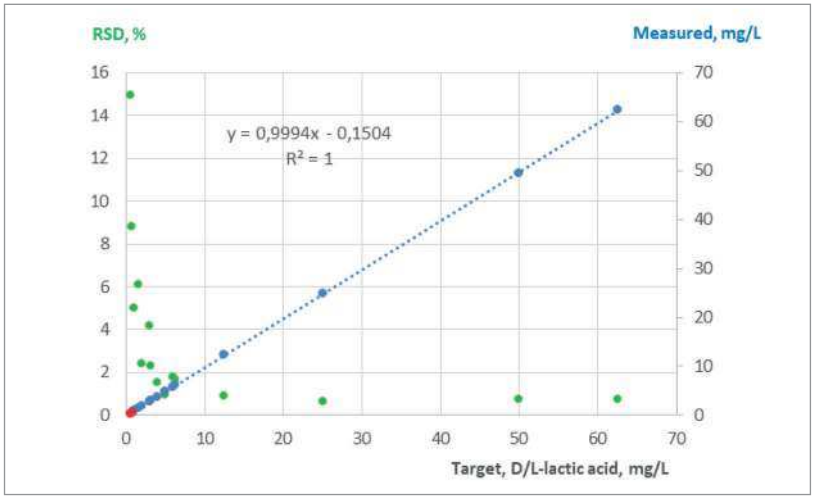

Figure 6: Characterization of linearity for the Sensitive range application with 100 µL sample volume; the red dots were not included in linear regression.

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4.4 Precision and recovery

The precision of the automated pipetting was characterized for all three application. Due to the different measurement range of each application, different dilutions of samples had to be used. In most cases, aqueous solution were used because the characterization of different matrices was already done during the validation of the manual application. To check for trueness one standard wine was applied for all three applications.

Table 1 shows the results for the Basic range application with a sample volume of 10 µL. As expected for automated pipetting RSDs at or below 1 % were obtained for concentrations between 300 mg/L and 500 mg/L. The validity of the four-point calibration was also checked with these four solutions. Recoveries ranged between 100 % and 104 % and were thus clearly within specifications.

Table 1: Characterization of precision for the Basic range (10 µL sample volume) application using three aqueous solutions and one reference wine.

	Aqueous solution			Reference wine
Replicate	Target: 450 mg/L	Target: 300 mg/L	Target: 500 mg/L	Target: 304 mg/L
1	464.0	311.8	501.4	301.5
2	460.5	312.9	504.5	302.8
3	468.9	314.0	504.5	304.2
4	466.2	315.8	506.6	306.8
5	457.8	309.4	498.3	300.0
6	460.6	311.3	499.1	301.6
7	458.4	312.9	500.5	301.8
8	467.2	311.6	503.8	304.7
9	467.9	315.5	498.5	305.2
10	458.4	310.4	495.3	297.5
Mean, mg/L	463.0	312.6	501.2	302.6
SD, mg/L	4.32	2.10	3.55	2.72
RSD (%)	**0.93**	**0.67**	**0.71**	**0.90**
Recovery (%)	102.9	**104.2**	100.2	99.5

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Table 2 shows the results for the High range application with a sample volume of 2 µL. As expected for automated pipetting and the small volume, RSDs at or below 2 % were obtained for concentrations between 300 mg/L and 500 mg/L. The validity of the four-point calibration was also checked with the three solutions and the standard wine. Recoveries ranged between 101 % and 104 % and were thus clearly within specifications. The standard wine comes with a certificate so that trueness of the system was established for the application with the smallest volume of 2 µL.

Table 2: Characterization of precision for the High range (2 µL sample volume) application using three aqueous solutions and a standard wine sample.

	Aqueous solution			Reference wine
Replicate	**Target: 450 mg/L**	**Target: 300 mg/L**	**Target: 500 mg/L**	**Target: 304 mg/L**
1	467.9	315.9	518.9	312.7
2	466.4	314.4	510.2	313.6
3	467.5	312.1	516.3	316.0
4	453.2	307.1	505.0	312.3
5	457.9	306.9	501.3	313.3
6	456.6	309.2	498.8	312.4
7	457.3	313.2	504.2	320.8
8	467.2	311.8	506.4	319.2
9	458.8	296.2	495.1	312.5
10	460.5	305.9	497.9	313.7
Mean, mg/L	461.3	309.3	505.4	314.7
SD, mg/L	5.41	5.70	7.80	3.06
RSD (%)	**1.17**	**1.84**	**1.54**	**0.97**
Recovery (%)	102.5	**103.1**	101.1	103.5

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Table 3: Characterization of precision for the Sensitive range (100 µL sample volume) application using three aqueous solutions and a reference wine.

	Aqueous solution			Reference wine
Replicate	**Target: 45 mg/L**	**Target: 30 mg/L**	**Target: 50 mg/L**	**Target: 30.4 mg/L**
1	45.28	29.84	48.73	32.00
2	45.51	29.88	48.98	32.24
3	45.45	30.05	49.01	32.26
4	45.85	30.21	49.39	32.41
5	45.72	30.20	49.27	32.44
6	45.19	29.83	48.76	32.08
7	45.32	29.91	48.84	32.17
8	45.46	29.92	48.93	32.10
9	45.66	30.09	49.26	32.40
10	45.76	30.05	49.34	32.35
Mean, mg/L	45.52	30.00	49.05	32.24
SD, mg/L	0.22	0.14	0.24	0.16
RSD (%)	**0.49**	**0.48**	**0.50**	**0.48**
Recovery (%)	101.2	**100.0**	98.1	106.1

Table 3 shows the results for the Sensitive range application with a sample volume of 100 µL. For this application a four-point calibration was validated. RSD values were at 0.5 % for concentrations between 30.4 mg/L and 50 mg/L. The validity of the four-point calibration was also checked with the four solutions. Recoveries ranged between 98 % and 106 %. The slighty higher recovery of the wine sample is maybe due to a high dilution of 1:100 to fit in the concentration range.

5. Conclusion

In summary, the data of the proof of concept show that the performance claims for food and beverages such as fermented milk and vegetable products, wines, beer, fruit juices, milk and egg are fulfilled. The method is robust and accurate for automated applications.