### **ORIGINAL ARTICLE**

## Automated assay of the potency of natural antioxidants using pipetting robot and spectrophotometry

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### Summary

In the food industry, in the process of creating new agricultural plant products, and in the testing of anti-cancer drugs there is often a need to assay multiple samples of low molecular weight antioxidants, plant samples and foods rich in antioxidants, with minimal additional costs and low degrees of uncertainty. With these demands in mind, we decided to study the fully automated assay of antioxidants using not only automated sample measurements but also automated processing of samples and application of reagents. The automated pipetting system epMotion 5075 and the automated spectrophotometer BS 400 were chosen for the assay purposes. Five methods were introduced for the automation: 2-diphenyl-1-picrylhydrazyl (DPPH) test, ferric reducing antioxidant power (FRAP) method, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) based test, N,N-dimethyl-1,4-diaminobenzene (DMPD) based test and the free radicals method. Samples containing one of the four antioxidants (standard rutin, quercitrin, ferulic and gallic acid) in a range  $1-1000 \ \mu g/ml$  were used throughout. All of the tested methods were found suitable for implementation in an automated assay. However, some of them, such as the ABTS test failed to assay all tested antioxidants. The coefficients of determination were also unequal. From the analytical point of view, FRAP methods provided the most reliable results in the automated assay; because of the capacity of the method, approximately 240 samples per hour (one sample per 15 seconds) can be assayed using the automated protocol. We were encouraged by the data received and we expect further interest in the practical performance of such automation. As a mean of testing the robustness of our method, in the next step of our study, oxidative status was assessed in model cell lines derived from prostate cancer (PC-3, PNT1A and 22RV1) that were cultured on ellipticine (0, 0.5, 1, 1.5, 2, 2.5, 5, 7.5, 10, 15 µmol/l) supplemented agar. Antioxidant activity was

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assessed (DPPH, ABTS, FRAP, DMPD, FR) and calculated on the phenolic antioxidant level (rutin, quercitrin, ferulic and gallic acid), and thus an estimation was formulated of the oxidative stress as a result of the impact of anti-cancer drugs. It can be demonstrated that the new method has wide applicability.

*Key words:* DPPH; FRAP; ABTS; DMPD; free radicals; antioxidant

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### Abbreviations

ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6- sulfonic acid;
DMPD, *N*,*N*-dimethyl-1,4-diaminobenzene;
DMSO, dimethyl sulfoxide;
DPPH, 2,2-diphenyl-1-picrylhydrazyl;
FeCl<sub>3</sub>, ferric chloride;
FR, free radical;
FRAP, ferric reducing antioxidant power;
FTB, foetal bovine serum;
TPTZ, 2,4,6-tripyridyl-s-triazine;
UV-VIS, ultraviolet-visible spectrum.

### INTRODUCTION

In a body, homeostasis, the maintenance of suitable inner conditions, is a primary task of many biochemical pathways. Equilibrium between the production of reactive oxygen (nitrogen) species and the ability to be protected from them is a physiological function of antioxidants. When antioxidants become depleted, oxidative stress can develop. Some pathological processes are consequences of the uncovered production of reactive oxygen and nitrogen species. Alzheimer's disease, Parkinson disease and other age related disorders are examples of the pathological processes, in which oxidative stress is suspected of playing a crucial role (Pohanka 2011). Alimental administration of antioxidants is considered as a way of preventing the pathological consequences of oxidative stress related dysfunctions. Plant extracts and plant enriched sources especially are considered suitable and easily available products for the prevention of these pathologies, as reported by e.g. Kaviarasan et al. (2008).

Unfortunately, research into low molecular weight antioxidants is not well standardized and differing protocols are used for the assay of antioxidants. Results can be influenced because of methodological errors as well as faults caused by human factors. The impact of methodological differences has been well reported. by e.g Müller et al. (2011), and the implementation of standard protocols with an automated assay procedure is not only suitable for simplification and cost saving but it is also necessary for the lessening of assay uncertainty and improvement of data validity. This experiment is aimed at the performance of a fully automated procedure suitable for fast and reliable assay of low molecular weight antioxidants in biological samples. The procedure uses fully automated manipulation with samples and reagents in order to receive a value with minimal cost and uncertainty.

### MATERIAL AND METHODS

#### Device and chemicals

The device used was composed of two basic parts: an automated pipetting system epMotion 5075 (Eppendorf, Germany) and an automated spectrophotometer BS 400 (Mindray, China). The pipetting provides a robotic arm with adapters (TS 50, TS 300 and TS 1000) and Gripper (TG-T). The empty microtubes are placed in the position B3 (scheme of depicted as Fig. 1) in adapter Ep0.5/1.5/2 ml. A Module Reservoir is located in the position B1, where stock solutions are available. The device is controlled by the epMotion control panel. Tips sized 300 and 1000  $\mu$ l (Eppendorf – Germany) are located in the A4 (ePtips 50), A3 (ePtips 300) and A2 (ePtips 1000) positions.

The automated spectrophotometer is composed of a cuvette space tempered to  $37\pm1$  °C, reagent space with a carousel for reagents (tempered to  $4\pm1$  °C), sample space with a carousel for preparation of samples and an optical detector. The transfer of samples and reagents is provided by a robotic arm equipped with a dosing needle (error of dosage up to 5% of volume). Cuvette contents are mixed by an automatic mixer, including a stirrer, immediately after the addition of reagents or samples. Contamination is reduced due to the rinsing system, which includes rinsing of the dosing needle as well as the stirrer by MilliQ water.

The chemicals used in this study include deionized water, rutin, quercitrin, ferulic and gallic acid, 2.2-diphenyl-1-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), 2.2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid (ABTS), potassium peroxodisulphate, 2,4,6-tripyridyl-s-triazine (TPTZ), hydrochloric acid, ferric chloride hexahydrate, sodium acetate trihydrate, *N*,*N*-dimethyl-1,4-diaminobenzene (DMPD), acetic acid and were purchased from Sigma Aldrich (St. Louis, MO, USA). The reaction buffer, chlorophyllin concentrate and its catalyst were purchased from Sedium R&D (Czech Republic).

### Standard

As standards, rutin, quercitrin, ferulic and gallic acid in a calibration range: 1; 2; 3; 4; 5; 6; 7; 8; 9; 10; 12.5; 15; 17.5; 20; 25; 30; 40; 50; 60; 70; 80; 90; 100; 125; 150; 175; 200; 250; 300; 350; 400; 450; 500; 750; 1000  $\mu$ g/ml were used. The standard calibration solutions were achieved by automated dilution of stock solutions 1000, 100 and 10  $\mu$ g/ml using deionized water.



**B**2

Tube\_2

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Fig. 1. epMotion 5075 automated pipetting system from frontal part.

**B1** 

TEMP1

Thermo\_1

# Determination of antioxidant activity by the DPPH test

B0

Waste

The DPPH test was used in compliance with a paper by Parejo et al. (2000). The method is based on the ability of the DPPH reagent to react with hydrogen donors.

Reagent preparation: 0.95 mmol/ml solution of radical DPPH was prepared by dissolving DPPH in 50 ml of DMSO followed by mixing with 100 ml of deionized water. The solution was kept in a dark flask and could be used for 7 days when stored at 4 °C.

Measurement procedure using the automated device: A 150  $\mu$ l volume of reagent is incubated with 15  $\mu$ l of sample. Absorbance was measured at 505 nm for 12 minutes, and the output ratio was established by calculating the difference between absorbance at the last (12<sup>th</sup>) minute and the second minute of the assay procedure.

# Determination of antioxidant activity by the ABTS test

The ABTS method is based on neutralization of a radical-cation arising from the one-electron oxidation of the chromophore 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic) acid. The reaction can be simply monitored spectrophotometrically (Re et al. 1999).

Reagent preparation: 7 mmol/l ABTS and 4.95 mmol/l potassium were mixed and dissolved in deionized water. The solution was then diluted with

deionized water in a ratio of 1:9 v/v. The solution was incubated for 12 hours in the dark and the reagent was kept suitable in the dark at 4 °C for one week.

**B4** 

C4

**B**3

TEMP3

Measurement procedure for an automated analyser: A 150  $\mu$ l volume of reagent was poured with 3  $\mu$ l of sample. Absorbance was measured at 660 nm. Antioxidant activity was calculated as the difference between absorbance at the last (12<sup>th</sup>) minute and at the second minute of the assay procedure.

# Determination of antioxidant activity by the FRAP method

The ferric reducing antioxidant power (FRAP) method is based on the reduction of ferric to ferrous salt by antioxidants and the following reaction of ferrous salt with 2,4,6-tripyridyl-*s*-triazine (TPTZ) reagent providing contrast blue (Ou et al. 2002).

Reagent preparation: Solution 1: 10 mmol/l solution of TPTZ, in 40 mmol/l of hydrochloric acid. Solution 2: 20 mmol/l solution of ferric chloride hexahydrate in deionized water. Solution 3: 20 mmol/l acetate buffer, pH 3.6 (weight of sodium acetate trihydrate was 0.272 g in 100 ml deionized water, adjusted by HCl). These three solutions (TPTZ, FeCl<sub>3</sub>, acetate buffer) are poured in a 1:1:10 ratio. The reagent could be used for seven days if stored at 4 °C in the dark.

Measurement procedure for an automated analyser: A 150 µl volume of reagent is injected into

a plastic cuvette with the subsequent addition of a 3  $\mu$ l sample (gallic acid solution). Absorbance is measured at 605 nm for 12 minutes. The difference between absorbance at the last (12<sup>th</sup>) minute and second minute of the assay procedure was used for calculating the antioxidant activity.

## Determination of antioxidant activity by the DMPD method

The compound *N*,*N*-dimethyl-1,4-diaminobenzene (DMPD) was converted to a relatively suitable and coloured radical form in the course of the ferric salt action. After addition of the antioxidant solution, the coloured solution of DMPD reagent was decolorized (Gulcin 2008, Gulcin et al. 2010).

Reagent preparation: Solution 1: acetate buffer (0.2 mol/l, pH 5.25); 1a) 2.17 g of sodium acetate trihydrate was dissolved in 80 ml of deionized water; 1b) 300 µl of concentrated acetic acid (>99.5 %, v/v) was diluted to a volume of 20 ml with deionized water. Shortly after mixing, pH = 5.5 was spontaneously reached. Solution 2: 0.74 mmol/l of ferric chloride: 1 mg of ferric chloride hexahydrate was dissolved with deionized water to a volume of 5 ml. Solution 3: 36.7 mmol/l DMPD: 25 mg of DMPD was dissolved in 5 ml of deionized water. The reagents were used immediately and prepared fresh for each day. Solutions 1,2 and 3 were mixed in a 20:1:1 (v/v/v) ratio.

Measurement procedure for an automated analyser: A 160  $\mu$ l volume of reagent was injected into a plastic cuvette with the subsequent addition of 4  $\mu$ l sample. Absorbance was measured at 505 nm. The difference between absorbance at the last (12<sup>th</sup>) minute and second minute of the assay procedure was used for calculating the antioxidant activity.

# Determination of antioxidant activity by the FR method

This method was based on ability of chlorophyllin (the sodium-copper salt of chlorophyll) to accept and donate electrons. This effect was conditioned by an alkaline environment and the addition of a catalyst and it was followed by a strong change in the absorbance maximum (Votruba et al. 1999).

Reagent preparation: 5 ml of reaction buffer (100 mmol/l of hydrochloric acid) was diluted with 45 ml deionized water. After that, 100  $\mu$ l of chlorophyllin was added. Once it became solved, 0.25 ml of catalyst was added. Reaction buffer was suitable for one month when stored at 2–8 °C in the dark.

Measurement procedure for an automated analyser: A 150  $\mu$ l volume of reagent is injected into a plastic cuvette with the subsequent addition of a 6  $\mu$ l sample. Absorbance was measured at 450 nm for the second minute of assay and the last (12<sup>th</sup>) minute. The difference between the two absorbencies was considered as an outputting value.

### Cell line experiments

In our study, we used the following prostatic cell lines PC-3, PNT1A, and 22RV1. The PC-3 was derived from the 4<sup>th</sup> level of prostatic adenocarcinoma. The PNT1A is a cell line derived from human prostatic epithelial cells. The last, 22RV1, is a human epithelial cell line derived from an epithelial graft of the cancer tissue. Culturing took place in flasks with surface 100 cm<sup>2</sup> and lasted 72 hours. Cell growth media HAM's F12 with 7% FVS for PC-3, RPMI-1640 with 10% FBS for 22RV1 and PNT1A were used. Ellipticine was dosed in the cell growth media up to level (0, 0.5, 1, 1.5, 2, 2.5, 5, 7.5, 10 and 15 µmol/l). The cultured cell lines were washed twice by PBS (6 ml of PBS and centrifugation on 2,700 RPM at 4 °C for 10 minutes). Supernatant was collected and used for assay of antioxidants in as described above.

### **RESULTS AND DISCUSSION**

# Calibration of the methods for antioxidant activity determination

The five methods described in the experimental chapter were used for the assay purposes. A summary of the suitability of the methods for assay of low molecular weight antioxidants used throughout experiments is depicted in Tables 1-4. The ABTS test, the DPPH test and FRAP method were suitable for assay of all the tested antioxidants. When regression of calibration curves carried out, good coefficients of determination were found for the three methods. The free radicals method was found unsuitable for assay of ferulic acid. The last method, DMPD, was found suitable for the assay of ferulic acid and gallic acid only, but the quercitrin assay by DMPD test was limited in comparison to the other methods. The low relevancy of DMPD is quite surprising as it has been used in previous experiments without any difficulties for assay of low molecular weight antioxidants in apricot samples (Sochor et al. 2011). Moreover, no crucial disadvantages were recognized when the method was tested on a trolox model (Sochor et al. 2010). The reason for the ineffectiveness of the DMPD test is not clear, but the low affinity of quercitrin and rutin toward the DMPD reagent and the equilibrium shifted to reactants can be inferred.

We selected the following standards - rutin, quercitrin, ferulic and gallic acid - to test the

Method	Wavelength [nm]	Measuring range [µg/ml]	Calibration equation	Confidence coefficient [R <sup>2</sup> ]	Standard deviation [%]
DPPH	505	1–17.5	y = -0.1533x - 1.6252	0.9945	1.96
ABTS	660	1–25	y = -0.071x - 0.9841	0.9977	2.26
FRAP	605	1–350	y = 0.0978x + 0.9774	0.9989	1.19
DMPD	505	1–30	y = -0.0509x - 0.2913	0.9982	2.68
FR	450	1–750	y = 0.0029x - 0.085	0.9991	1.11

Table 1. Summary of the parameters for individual methods related to the standard gallic acid.

Table 2. Summary of the parameters for individual methods related to the standard ferulic acid.

Method	Wavelength [nm]	Measuring range [µg/ml]	Calibration equation	Confidence coefficient [R <sup>2</sup> ]	Standard deviation [%]
DPPH	505	1–100	y = -0.0548x - 1.1467	0.9927	1.53
ABTS	660	1–150	y = -0.038x - 0.7683	0.9915	2.28
FRAP	605	1–175	y = 0.0284x + 0.2702	0.9973	1.42
DMPD	505	17.5-800	$y = -0.458 \ln(x) + 0.1955$	0.9852	2.31
FR	450	_	_	_	_

Table 3. Summary of the parameters for individual methods related to the standard quercitrin.

Method	Wavelength [nm]	Measuring range [µg/ml]	Calibration equation	Confidence coefficient [R <sup>2</sup> ]	Standard deviation [%]
DPPH	505	7–70	y = -0.0484x - 1.3425	0.9992	1.63
ABTS	660	7–70	y = -0.0309x - 1.1151	0.9959	2.03
FRAP	605	1–1000	y = 0.0342x + 0.4564	0.9996	1.02
DMPD	505	_	_	_	_
FR	450	1-1000	y = 0.0039x + 0.127	0.9996	1.27

automation of methods for determination of antioxidant activity. All tested molecules can be used as a standard for calibration of antioxidant assays and their selection mainly depends on the type of material analysed and the laboratory equipment used. However, automated spectrometric assays tested in this study must be effective with all these substances to show the versatility of the system. Ferulic acid was the first compound tested (Fig. 2). The dependencies of absorbance on the concentration of ferulic acid measured by the DPPH test, ABTS test, FRAP method, DMPD method, and the free radicals method are shown in Figs 2A, B, C, D and E. The structure of gallic acid is shown in Fig. 2F. The same results were obtained for ferulic acid (Fig. 3), quercitrin (Fig. 4) and rutin (Fig. 5).

Method	Wavelength [nm]	Measuring range [µg/ml]	Calibration equation	Confidence coefficient [R <sup>2</sup> ]	Standard deviation [%]
DPPH	505	1–25	y = -0.0354x - 0.3071	0.9966	1.15
ABTS	660	60–1000	y = -0.0053x - 0.5518	0.9982	2.08
FRAP	605	1–175	y = 0.0212x + 0.3304	0.9983	1.28
DMPD	505	_	_	_	-
FR	450	1-1000	y = 0.0039x - 0.1429	0.9994	1.01

Table 4. Summary of the parameters for individual methods related to the standard rutin.



Fig. 2. Assay of gallic acid using the DPPH test (A), the ABTS test (B), the FRAP method (C), the DMPD method (D), and the FR method (E). The last part of figure is the structure of gallic acid (F).

Calibration plots for the tested methods are shown in Figs 2–5. We found good coefficients of determinations in a range 0.9927-0.9992 for the DPPH test, 0.9915-0.9982 for the ABTS test, 0.9973-0.9996 for the FRAP method, 0.9852 and 0.9982 for the DMPD method and the two successful calibrations, and 0.9991-0.9996 for the free radicals method and the three successful calibrations. It clearly follows from the results obtained that the best correlations were found for the FRAP and free radicals methods thus favourable adaptation to automation can be inferred.

The calibrations shown had differing linear ranges. The FRAP and free radicals methods providing linear calibration as high as  $1,000 \mu g/ml$  for some antioxidants, had the longest linear ranges, but



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Fig. 3. Assay of ferulic acid using the DPPH test (A), the ABTS test (B), the FRAP method (C), the DMPD method (D), and the FR method (E). The last part of figure is the structure of ferulic acid (F).



Fig. 4. Assay of quercitrin using the DPPH test (A), the ABTS test (B), the FRAP method (C), the DMPD method (D), and the FR method (E). The last part of figure is structure of quercitrin (F).



Fig. 5. Assay of rutin using DPPH test (A), ABTS test (B), FRAP method (C), DMPD method (D), FR method (E). The last part of figure is structure of rutin (F).

in contrast, the DPPH test was limited by approximately 100  $\mu$ g/ml for the ferulic acid assay and even 20  $\mu$ g/ml for gallic acid. The good results for the FRAP method are not surprising considering its applicability for low molecular weight antioxidants assays in tissue samples (Pohanka et al. 2011), assessment of antioxidants in fruit (Bouayed et al. 2010), and *in vitro* assessment of antioxidants such as uric acid assay (Duplancic et al. 2011). Considering the automation process, the lengths of linear ranges are not dependent on automation but on the reaction principle and molecular mechanism of methods pre-limiting them for applicability in the automation of assays.

The major advantage of the automated assay is the short time needed per sample. Though the total time from inputting the sample to receiving the measured value is nearly a quarter of an hour, the total time per sample is less than one minute. When the automated device is used for an extensive series of samples, the total time per sample is approximately 15 seconds. It means that 240 samples can be assayed per hour by one selected method without any additional effort or special need for skilled manipulation of the samples.

The performance of an automated pipetting device with automated spectrophotometer is suitable for a fast and reliable assay of low molecular weight antioxidants. The automated pipetting system epMotion 5075 and the automated spectrophotometer BS 400 were both suitable for the assay purposes and all of the selected experimental protocols were adaptable for analytical purposes. The failure in the assay of rutin by the DMPD method, and in the assay of ferulic acid by the free radicals method was not caused by the inapplicability of the automated assay but by shortcomings in the applicability of protocols for some compounds. This was not disclosed in the databases searched. The easy availability of the DMPD method has been reported in the work of Damien Dorman et al. (2011). On the other hand, samples are commonly assayed by one or two methods, so discrepancies in natural sample assays can stay hidden. When the performance of the automated protocol is considered using all of the described methods, the FRAP method meets the best criteria as it was successfully performed for all antioxidants, it has a long linear range and good coefficients of determinations. On the other hand, no



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Fig. 6. Assayed antioxidants in cell lines using the DPPH test (A), the ABTS test (B), the FRAP method (C), the DMPD method (D), and the FR method (E). Calculated on gallic acid.

serious reason arose to discriminate against the other methods and the last four protocols are applicable for the construction of a fully automated protocol also.

The significance of low molecular weight antioxidants in biological samples has been described by several investigators (Kaviarasan et al. 2008, Salehi et al. 2012). The methods proposed here can be used for the fast and reliable processing of natural samples to judge their antioxidant capacity. When the automated pipetting system and the automated spectrophotometer are used simultaneously, natural samples can be assessed with good effectiveness and low uncertainty.

# Application of the optimized methods on real sample analyses

The test for suitability of the automated assay for evaluation of antioxidants in cell lines was performed in prostatic cancer cells exposed to ellipticine. The drug damages the DNA of cancer cells and stops transcription and replication. The cells are not able to divide, and as a result, the cells are directed to apoptosis (Stiborová et al. 2011, Kizek et al. 2012). However, the drug does not act specifically on cancer cells and normal cells can be affected, too. The aim of our experiment was to estimate the scale of oxidative stress – expressed as the antioxidant activity – in



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Fig. 7. Assayed antioxidants in cell lines using the DPPH test (A), the ABTS test (B), the FRAP method (C), the DMPD method (D), and the FR method (E). Calculated on ferulic acid.



Fig. 8. Assayed antioxidants in cell lines using the DPPH test (A), the ABTS test (B), the FRAP method (C), the DMPD method (D), and the free radicals method (E). Calculated on quercitrin.



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Fig. 9. Assayed antioxidants in cell lines using the DPPH test (A), the ABTS test (B), the FRAP method (C), the DMPD method (D), and the FR method (E). Calculated on rutin.

cancer cells and cells affected by a cytostatic drug. The experiment was aimed at a comparison of the effect of ellipticine on cancer and normal cell lines. The results are depicted in Figs 6-9. We used all of the optimized methods - DPPH, ABTS, FRAP, DMPD and free radicals- for determination of the antioxidant activity. Primarily, we re-calculated the results on the gallic acid, which is the most commonly accepted standard for calibration of the assay (Fig. 6). From the methodological point of view, it clearly follows from the results obtained that all methods gave similar results, i.e. that increasing the dose of ellipticine resulted in the decrease of antioxidant activity. The radicals measured in the real samples are shown in Fig. 6F. Further, we re-calculated the results obtained on ferulic acid (Fig. 7). As was shown above, ferulic acid is not a suitable standard for calibration of the free radicals assay. Therefore, we did not use this re-calculation. The rest of the methods gave similar results to gallic acid. In the case of re-calculation on quercitrin (Fig. 8) and rutin (Fig. 9), we did not use the results obtained from the DMPD assay.

From the biological point of view, the depletion of antioxidants was more striking when the ABTS test and FRAP were used for assay purposes. The last two tests were less sensitive. The depletion of antioxidants scaled by the DMPD and free radical methods proved less extensive alterations in comparison with the ABTS test and FRAP; in spite of the fact that ellipticine is able to generate reactive oxygen species, the antioxidant capacity decreased. This phenomenon is related to the fact that the drug used was cytotoxic. Therefore, the antioxidant capacity clearly correlated with the viability of the cells. Interestingly, we did not confirm the differences between the types of cell line used in our experiment. These results could be related to the fact that ellipticine is toxic for prostate cells equally from the point of view of generation of reactive oxygen species, which has to be considered prior to use of this drug for the treatment of this cancer. Besides, the transformation of a healthy prostate cell line into a tumour did not influence the sensitivity to antioxidant mechanism in a cell. Toxicity of anticancer drugs is widely discussed in scientific works (Zanella et al. 2011, Zong et al. 2011). Oxidative stress, as a consequence of the impact of the drugs is not adequately investigated, as elaborative protocols are needed for the assay. Understanding of the oxidative stress related processes in isolated conditions is necessary when the effect of a new drug is researched.

### CONCLUSIONS

An automated method for the fast and reliable assay of antioxidants was developed. The method is suitable for laboratory estimation of antioxidant potency in biological samples. Though five methods, ABTS test, DPPH test, FRAP method, DMPD test and free radicals method, were successfully performed, their suitability for automated assay was unequal. From the methods tested, FRAP provided the most favourable parameters for implementation into the automated device. Antioxidant activities in cells exposed to ellipticine decreased in a dose dependent manner. We can infer the contribution of the anticancer drug to oxidative damage in cells and compare the effect on normal as well as cancer cells. The method performed is suitable for these purposes.

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