Capillary Electrophoresis with Laser-induced Fluorescence Detection for Application in Intracellular Investigation of Anthracyclines and Multidrug Resistance Proteins

Julius Mbuna*† and Takashi Kaneta**

*Dar es Salaam University College of Education, Department of Chemistry, P.O. Box 2329, Dar es Salaam, Tanzania
**Department of Chemistry, Graduate School of Natural Science and Technology, Okayama University, Okayama 700-8530, Japan

Capillary electrophoresis (CE) coupled with laser-induced fluorescence (LIF) is a powerful method for the trace analysis of cellular components. This review presents a summary of topics for the direct analysis of anthracyclines and multidrug resistance proteins in cancerous cells. A micellar electrokinetic chromatography (MEKC) method that does not use organic solvents, and hence prevents the precipitation of proteins in cellular samples, was shown to be a reliable method for the determination of several anthracyclines including the epimeric doxorubicin and epirubicin. A fast CE-based immunoassay for investigating transporter multidrug resistance associated protein (MRP1) was also developed for routine or explorative analysis of the levels of transporter proteins in cancerous cells. A combination of the developed MEKC-LIF method and the CE immunoassay (CEIA) method has permitted the analysis of anthracyclines and MRP1 in a cell line to show the relationship between the levels of MRP1 and amount of anthracyclines in cancerous cells.

Keywords Capillary electrophoresis, micellar electrokinetic chromatography, capillary electrophoresis immunoassay, laser-induced fluorescence, anthracycline, multidrug resistance associated protein, cancer cell

(Received June 17, 2015; Accepted August 5, 2015; Published November 10, 2015)

1 Introduction

Cancer is a growing, global health problem with a larger proportion of new cases reported in low-to-medium income countries than in high-income countries. The World Health Organization (WHO) projects the global cancer incidence to rise from 14 million in 2012 to 22 million in 2032, with more than 70% of cancer deaths occurring in central and south America, Africa, and Asia. Globally, cancer cases are expected to rise,
transporter proteins,15 reduced drug uptake,16 the activation of complex matrices, including urine,11 serum,12 plasma13 and of various chemical species in cancerous cells.

MDR can be mediated by the active efflux of the drugs by multidrug resistance proteins, which has been reported19 as impossible.

Various methods for the determination of anthracyclines have been reported, including amperometry,6 laser flow cytometry,7 polarography,3 ultraviolet-visible spectroscopy,9 and voltammetry.10 It is worth noting that measurements of this important class of anticancer drugs can be facilitated by their native fluorescence, which allows trace and ultra-trace determinations in a range of matrices. Most high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) have exploited this intrinsic property to determine the levels of anthracyclines in complex matrices, including urine,11 serum,12 plasma13 and individual cells.14

Like any xenobiotic, anticancer drugs elicit multidrug resistance response from cancerous as well as non-cancerous cells. Multidrug resistance (MDR) is a phenomenon in which cells develop resistance over chemically diverse and unrelated drugs, whereby drugs are constantly effluxed from the cell. MDR can be mediated by the active efflux of the drugs by transporter proteins,15 reduced drug uptake,16 the activation of regulated detoxifying systems, such as cytochrome P450 mixed-function oxidases,17 and defective apoptotic pathways. Since cancer cells gain MDR through several mechanisms,18 we need to develop methods by which we can study different aspects of this phenomenon, especially for the measurement of multidrug resistance proteins, which has been reported19 as being crucial in MDR mediation. Such multidrug resistance proteins can be labeled with fluorescent dye, and subsequently be determined by fluorescent techniques.

Ever since its introduction in CE by Terabe et al.,20 micellar electrokinetic chromatography (MEKC) has remained a powerful tool in the analysis of both neutral and charged organic molecules21–22 as well as macromolecules including proteins and peptides,23 carbohydrates,24 nucleic acids.25 When combined with laser-induced fluorescence (LIF), MEKC becomes a highly sensitive method for the determination of trace amounts of analytes in increasingly complex matrices. Thus, MEKC-LIF and CE-LIF can be applied to determine both anthracyclines and multidrug resistance proteins as well as to study their interactions in the use of inhibitors to control MDR in cancer cells. MEKC-LIF, therefore, has been applied to the determination of analytes in biological systems, especially the in vitro determination of extogeneous as well as endogeneous chemical species.26–28

This review examines the use of MEKC-LIF in the determination of anthracyclines, which are known anticancer agents, and one group of cell proteins (multidrug resistance associated protein, MRPI) that is responsible for efflux xenobiotics including anticancer agents, covering research work done in Kyushu University’s Applied Chemistry Department over a period of three years (2008 - 2011).

2 Direct Determination of Anthracyclines by MEKC-LIF

Since anthracyclines have been analyzed for over three decades, various analytical methods are available for determinations in various matrices.6–10 These methods have shown individual strengths and limitations. For instance, polargraphy,4 despite its cheap instrumentation, involves the extraction of doxorubicin and daunorubicin from urine or plasma into chloroform before any determination. The use of organic solvent will not only precipitate proteins, but chloroform is itself a banned solvent in many countries. While voltammetric determinations of anthracyclines are sufficiently sensitive, this process exhibits poor resolution of anthracyclines with similar structures. Other methods, like flow cytometry40 with an advantage of being invaluable for non-invasive determination of levels of anthracyclines, require expensive instrumentation to carry out the analysis.

The bulk of anthracyclines determinations are done by either capillary electrophoretic3–14,20–25 or chromatographic11,12,29–33 techniques coupled with various detectors, including UV, LIF and mass spectrometry. Chromatography has several advantages, including superior sensitivity but requires large amounts of solvents, expensive columns and equipment, comparatively larger sample size and the extraction of analytes. HPLC has been used to determine anthracyclines, including doxorubicin (DOX), epirubicin (EPI), idarubicin (IDA) and daunorubicin (DNR), in various matrices,34–36 in which extraction using organic solvent was used to obtain the anthracyclines or corresponding metabolites from the matrices. A case in point is the HPLC-fluorescence detection of DOX, EPI, DNR, IDA and their metabolites from plasma and saliva after liquid-liquid extraction.37 The use of organic solvents inevitably leads to the precipitation of proteins in the sample making direct measurements of proteins in the same sample impossible.

Compared to HPLC techniques, CE determinations are credited with various advantages, including small size of the analytes, cheaper instrumentation, and direct analysis of the sample precluding any possibility of degradation. CE measurements by Reinhold et al.38 determined the levels of EPI and DOX by CE-LIF in which the migration buffer contained a high proportion (70% (v/v)) of acetonitrile. A migration solution containing 30% (v/v) acetonitrile was used in a similar separation of DOX, IDA and DNR29 using CE-LIF. Separations of DOX and DNR after extraction, were reported by Simeon et al.40 and Hempel et al.41 in the determination of anthracyclines in plasma and tumors. Similar work employing cyclodextrin (CD)-assisted MEKC on the separation of DOX and doxorubicinol was reported by Eder et al.42 It should be mentioned that the aforementioned CE methods used organic solvents, which are known to precipitate proteins in biological samples, either during separation or extraction. To separate
analytes without the precipitation of proteins requires a purely aqueous method. Such methods are extremely useful, since they can be used in the determination of cellular proteins including the multidrug transporter proteins generated in cells.

An entirely aqueous, CE method facilitates the measurements of anthracyclines and proteins in the same sample solution, and hence permits simultaneous measurement of anthracyclines and proteins in a given sample solution. While MEKC determination of DOX in the presence of other anthracyclines like IDA and DNR is less demanding because of some basic structural differences, the separation of DOX from EPI is challenging because the two are epimers, only differing in the axial and equatorial orientations of the OH group at C-4 in daunosamine sugar moiety (Fig. 1). Developing a direct, aqueous MEKC method for anthracyclines determination is not only useful in intracellular determination of anthracyclines, but will also find applications in the quantification of similar agents, especially in hospital wastewater involved in environmental investigations. Because of their native fluorescence, LIF is an ideal detector. For direct MEKC-LIF determination, a lab assembled setup, equipped with an argon-ion laser emitting at 488 nm, was employed. The migration solution used to achieve the separation of anthracyclines is made up of 20 mM sodium tetraborate buffer containing 35 mM sodium taurodeoxycholate (STDC), 3.5% (wt/v) (2-hydroxypropyl)-γ-CD (HP-CD), and 20 mM sodium dodecyl sulfate (SDS). Two chiral selectors, HP-CD and STDC, enhance the separation selectivity between DOX and EPI, whereas SDS was needed to separate an internal standard from the analytes. This MEKC-LIF method has been shown to be useful in the base-line separation of DOX and EPI (Figs. 2(A) and 2(B)) or DOX, EPI, IDA and DAU (Fig. 2(C)) contained in cancerous cells. The reliability of the MEKC method is displayed by Table 1, where the relative standard deviations for intraday and interday were lower than 3.7 and 5.42%, respectively. The LOD of the anthracyclines determined

Fig. 1 Structures of (A) doxorubicin (DOX) (B) epirubicin (EPI) (C) daunorubicin (DNR) and (D) idarubicin (IDA).

Fig. 2 Separation of anthracyclines under optimized conditions. 1. EPI; 2. DOX; 3. IDA; 4. DNR; 5. rhodamine B. (A) Standard mixture of 500 nM EPI, 500 nM DOX, and 4.5 nM rhodamine B, (B) A549 cell lysate treated with DMEM medium containing 500 nM EPI and 500 nM DOX (Lysis buffer contained 4.5 nM rhodamine B), (C) standard mixture of 300 nM of EPI, DOX, DAU, DNR, and 4.5 nM rhodamine B. Migration buffer: 20 mM sodium tetraborate buffer containing 35 mM STDC, 3.5% (wt/v) (2-hydroxypropyl)-γ-CD (HP-CD), and 20 mM sodium dodecyl sulfate (SDS); separation voltage, 15 kV; Injection 10 s under gravity (vial raised 5 cm above outlet vial). Reproduced from Refs. 26 and 43 with permission from John Wiley and Sons.
by CE-LIF lies in the ng mL\(^{-1}\) range. It is worth noting that the LOD of DOX (4.9 ng mL\(^{-1}\)), EPI (4.8 ng mL\(^{-1}\)), IDA (2 ng mL\(^{-1}\)) and DAU (1.3 ng mL\(^{-1}\)), measured in aqueous buffers, is comparable with the LOD determined in an aqueous-methanol buffer (10 ng mL\(^{-1}\)), but slightly higher than those determined in aqueous-acetonitrile buffer (1 ng mL\(^{-1}\)). The CE-LIF quantitation range of the anthracyclines determined in aqueous buffer is comparable with that measured in aqueous-acetonitrile buffer (10 – 500 ng mL\(^{-1}\)). Because of the incompatibility of buffers used in anthracyclines analysis with mass spectrometry, a comparison with LC/MS is informative. The LOD for DOX, determined by LC-ESI-MS/MS (1 ng mL\(^{-1}\)) is comparable with CE-LIF. However, the quantitation range of DOX determined by LC-ESI-MS/MS (10 – 100 ng mL\(^{-1}\)) is narrow compared to that determined by LIF-CE. By the CE-LIF method employing aqueous buffer, the influx of anthracyclines in cancerous cells as well as accumulation can be monitored continuously over a wide time range.

The influx of xenobiotics in a living cell naturally triggers a cellular response to efflux the xenobiotic agent. Proteins in the cell membrane are known to be responsible for the efflux of a wide variety of structurally-unrelated drugs, thereby causing

| Analyte | Concentration/ nM | Recovery\(a\), % ± SD | Precision; % RSD | LOD/ nM |
|---------|-------------------|------------------------|------------------|--------|
| EPI     | 50                | 101.2 ± 0.4            | 2.0              | 8.77   |
|         | 150               | 101.5 ± 0.5            | 2.5              | 5.1    |
|         | 250               | 99.2 ± 0.9             | 3.7              | 5.2    |
| DOX     | 50                | 100.3 ± 0.5            | 3.6              | 4.8    | 9.12   |
|         | 150               | 99.4 ± 0.4             | 3.1              | 3.9    |
|         | 250               | 99.0 ± 0.9             | 3.1              | 4.1    |
| IDA     | 50                | 97.8 ± 0.7             | 1.2              | 3.6    | 4.11   |
|         | 150               | 99.5 ± 0.8             | 2.6              | 4.7    |
|         | 250               | 98.4 ± 0.6             | 1.9              | 4.4    |
| DAU     | 50                | 98.1 ± 0.9             | 1.6              | 4.1    | 2.42   |
|         | 150               | 99.5 ± 0.9             | 2.8              | 4.2    |
|         | 250               | 100.9 ± 0.7            | 2.5              | 5.4    |

\(a\). \(n = 7\); Linear range: 25 nM - 1 μM. Reprinted from Ref. 43 with permission from John Wiley and Sons.

Fig. 3 Accumulation of anthracyclines in RDES cells following incubation with inhibitors. White bar, 12 hours-incubation; black bar, 24 hours-incubation. Cells were cultured in the medium containing four anthracyclines and inhibitor(s) (probenecid and verapamil). Simultaneous treatment was employed for all cell samples. Inh F, inhibitor free; P, 10 μM probenecid; V, 10 μM verapamil; PV, a mixture of 10 μM probenecid and 10 μM verapamil. Reproduced from Ref. 43 with permission from John Wiley and Sons.
MDR. Since cancer cells are particularly effective in effluxing the xenobiotics, we need a strategy to increase the cellular accumulation of drugs in order to realize the objective of chemotherapy, i.e. the death of the cancer cells. The use of inhibitors including verapamil, probenecid or cyclosporins may increase the accumulation of anticancer drugs. The MEK-LIF method can be used to follow the in vitro accumulation of different anthracyclines upon the incubation of cells in the presence of single or a combination of inhibitors. For instance, the presence of inhibitors in Ewing’s sarcoma tumor cells (RDES cells) generally increase the accumulation of anthracyclines, albeit to different extents (Fig. 3). It can also be inferred from Fig. 3 that the combination of inhibitors used does not result in any significant advantage over a single inhibitor for RDES cells.

3 CEIA with LIF for the Determination of MRP1

MDR, whether intrinsic or acquired, is responsible for the relapse of chemotherapy treatments of cancer. Although several factors lead to MDR, the efflux of drugs by protein transporters constitute one of the key mechanisms giving rise to MDR. As far as cancer cells are concerned, these multidrug transporter proteins belong to two main categories, namely adenosine triphosphate (ATP)-binding cassette (ABC) and lung resistance-related proteins (LRP). These multidrug transporter proteins render chemotherapy treatments ineffective by reducing the intracellular concentration of drugs through active efflux, thereby allowing cancer cells to avoid death. The ABC superfamily is a more dominant category of transporter proteins, which is made up of P-glycoproteins, multidrug resistance-associated proteins (MRP), and breast cancer-related proteins (BCRP). P-glycoproteins is the most extensively investigated transporter proteins among the ABC superfamily, and is known to efflux a wide range hydrophobic xenobiotics. P-glycoprotein is widely expressed in many types of cancers including cancers of hematopoietic, urogenital, and gastrointestinal systems as well as childhood cancers. P-glycoprotein is mainly responsible for transporting neutral or positively-charged hydrophobic compounds. Conversely, the MRP family transports organic anions including, glutathione (GSH), glucuronate or sulfate conjugates of organic anions. BCRP effluxes large, hydrophobic (positively- or negatively-charged) molecules together with cytotoxic compounds (mitoxantrone, topotecan, flavopiridol, and methotrexate), and fluorescent dyes. While P-glycoprotein is the most extensively investigated, there is increasing interest in investigations of MRP and BCRP owing to their roles in conferring multidrug resistance in cancer cells. In order to study the effects of these proteins in MDR, quantitative analytical methods are required as an important aspect of such investigations.

Measurement of amounts of chemical species can be achieved by either relative or absolute quantification. The absolute quantification of any chemical species requires the availability of standards. For the determination of multidrug transporter proteins, absolute quantification is demanding because it requires complicated synthesis, purification and confirmation of the identity of the proteins as standards before quantification by a specific technique, like HPLC. It is noteworthy that relative quantification forms the basis of most methods used for the analysis of transporter proteins, including electrochemical immunoassay, PCR, Western blotting and flow cytometry. The aforementioned techniques are well established in immunoassay, exhibiting merits as well as limitations. The Electrochemical immunoassay has an advantage of high sensitivity. It, however, can only determine proteins on the surface of cells, i.e., the assay is ineffective for determining proteins in cell organelles. This weakness is also observed in flow cytometry, which also happens to be an expensive method. Similar to electrochemical immunoassay, PCR methods show high sensitivity, but suffer from a long analysis time and false positives due to ease of contamination during analysis.

Transporter proteins are located both in the plasma membrane and in the cell organelles like the nucleus, making it necessary to lyse the cell in order to fully quantify the proteins. Established methods, like Western blot, ELISA, and flow cytometry, can be employed for such analysis, but CEIA is a superior method, because it addresses the shortcoming of the aforementioned methods through ease of automation, small sample size, shorter analysis time, and multi-analyte capability. CEIA can be performed in either indirect or direct formats. In CEIA, aptmers, enzymes, and antibodies in competitive and noncompetitive formats interact with antigens to form immunocomplexes in highly complicated matrices. Reported studies include the determination of drugs and metabolites, hormones, toxins, proteins, and peptides. It is worth noting that most of the protein determinations by CEIA have involved low molecular weight proteins (10–90 kDa), rather than the higher molecular weight proteins (170 kDa), like the ABC transporter proteins.

Compared to absolute quantification, relative quantification can be useful for routine and preliminary examinations of analytes. One way to carry out relative quantification is to employ a noncompetitive CEIA method, in which LIF can be used for detection of the transporter protein. For this method to work, baseline resolution between the antibody labeled with a fluorescent tag (labeled antibody) and its immune complex with the analyte is a prerequisite. In this method, cell lysate was reacted with excess amounts of the labeled antibody (e.g. anti MRP1) in the presence of an internal standard, after which the mixture was immediately injected into the CE system to yield the peaks shown in Fig. 4(A). The result represents the electropherogram before the immunological reaction took place. When the mixture was incubated and injected into a CE system after suitable intervals, two peaks corresponding to the free uncomplexed antibody and its immune complex were observed in the electropherograms (Fig. 4, (B) – (E)). The amount of protein in the cell lysate can be determined from immune complex alone, and not from the residual amount of the free uncomplexed antibody after incubation.

If $P_{\text{A} \text{b},0}$ and $P_{\text{AIS,pre}}$ represent the peak area of free antibody and the internal standard before incubation, and $P_{\text{A} \text{b}, \text{post}}$ and $P_{\text{AIS,post}}$ represent the peak area of the immune complex and internal standard after incubation for some interval, then

$$C_{\text{A} \text{b},0} = \frac{P_{\text{A} \text{b},0}/P_{\text{AIS,pre}}}{P_{\text{A} \text{b}, \text{post}}/P_{\text{AIS,post}}},$$

where $C_{\text{A} \text{b},0}$ and $C_{\text{A} \text{b}, \text{post}}$ are the respective concentrations of antibody before incubation and the immune complex produced after some interval. Assuming that the complex formed consists of one antigen and one antibody, the concentration of the protein (MRP1), $C_{\text{MRP1}}$, is equal to $C_{\text{A} \text{b}, \text{post}}$ and is therefore calculated directly from

$$C_{\text{MRP1}} = \frac{C_{\text{A} \text{b},0}/P_{\text{A} \text{b},\text{pre}}}{P_{\text{A} \text{b}, \text{post}}/P_{\text{AIS,post}}}.$$

To correct for the number of cells analyzed, it is necessary to
1126 ANALYTICAL SCIENCES   NOVEMBER 2015, VOL. 31

determine the total amount of proteins in the cell sample by a suitable protein determination method, like the bicinchoninic acid assay. Thus, a simple relative measure of MRP1 can be determined as:

\[
C_{\text{MRP1}} = \left( \frac{C_{\text{protein}}}{PA_{\text{ab,0}}/PA_{\text{IS-pre,cplx}}} \right) \times \frac{PA_{\text{ab,0}}}{PA_{\text{IS-post}}}.
\]  (3)

where \(C_{\text{protein}}\) is the concentration of proteins in the cell sample and \(P_I\) is the total amount of protein in mg mL\(^{-1}\).

The CEIA shows potential for kinetic investigations of immunological reactions, since the peak areas of both the free antibody and the immune complex can be determined independently of time. Figure 5\(^{27}\) shows the variation in the peak area of the complex with time for an anti MRP1 antibody. The plot shows that the reaction is complete after about 1 h. Such measurements can be adapted for empirical kinetic studies of immune complexation.

Relative quantification has been applied to determining the amount MRP1 in cancerous cells, including lung cancer cells (A549) and RDES.\(^{27}\) As shown in Table 2, the amount of MRP1 increases significantly during the first 12 h after introducing a dose of doxorubicin into the culture medium, beyond which a modest change in MRP1 is noted. This general increase in MRP1 leads to a possible increase in the drug efflux and hence lowered concentration of the drug in the cell. In the presence of an inhibitor like probenecid, the amount of MRP1 generally increases with both the exposure time and the amount of the added inhibitor. An increase in the amount of the drug in the presence of a higher concentration of the inhibitor may suggest that the inhibitor, itself, is a substrate for MRP1, and hence being effluxed instead of doxorubicin. From the aforementioned results, the developed CEIA method can be used for measurement and hence monitoring of the total amount of MRP1 in cancerous and non-cancerous cells.

4 Conclusions

A purely aqueous MEKC-LIF method for the measurement of epimeric anthracyclines (DOX and EPI) in the presence of other anthracyclines is effective for the in vitro determination of anthracyclines levels in cells. This method can be extended to the determination of anthracyclines in complex environmental samples including investigations of anthracyclines in hospital waste water. The CEIA method for the relative determination of resistance transporter proteins, MRP1, using antibodies, addresses shortfalls of established techniques including flow

---

**Table 2** The levels of MRP1 expression and the amount of accumulated DOX in cancer cells

| Cell type | Treatment | Relative amount of MRP1/protein content/nM mg\(^{-1}\) | Amount of DOX/protein content/\(\mu\)M mg\(^{-1}\) |
|-----------|-----------|---------------------------------------------------|---------------------------------------------|
| A549      | F         | 76.4 ± 2.4                                         | 0                                           |
| A-12      | 98.8 ± 4.0 | 0.42                                              |                                             |
| A-24      | 94.0 ± 3.2 | 0.26                                              |                                             |
| AI-12     | 144 ± 5.8  | 0.99                                              |                                             |
| AI-24     | 90.0 ± 3.2 | —*                                                |                                             |
| RDES      | F         | 43 ± 2.0                                           | 0                                           |
| A-12      | 68.0 ± 3.0 | 1.15                                              |                                             |
| A-24      | 70.0 ± 2.0 | 0.99                                              |                                             |
| AI-12     | 98.0 ± 4.4 | 1.56                                              |                                             |
| AI-24     | 73.6 ± 2.6 | —*                                                |                                             |

F: DOX free, A-12. 12 h incubation with DOX, A-24. 24 h incubation with DOX. AI-12. 12 h incubation with DOX and probenecid. AI-24. 24 h incubation with DOX and probenecid. *Amounts of DOX were not determined for AI-24. Reprinted from Ref. 27 with permission from Elsevier.
cytometry and ELISA, owing to its speed, cost, and reliability; it can be used for both explorative and detailed investigations of resistance proteins in cancerous cells. The method can easily be adapted for measurement of other ABC protein transporter proteins, like P-glycoprotein and BCRP.

5 Acknowledgements

A part of the research was supported by the program of a JSPS postdoctoral fellowship for overseas researchers and Grants-in-Aid for Scientific Research, Scientific Research (B), from the Japan Society for the Promotion of Science. J. M. acknowledges Prof. Totaro Imasaka for his kind hosting and helpful discussion in research done at Kyushu University.

6 References

1. P. Vineis and C. P. Wild, Lancet, 2014, 383, 549.
2. WHO, Cancer fact sheet 297, http://www.who.int/mediacentre/factsheets/fs297/en/.
3. T. Hoppe-Tichy, J. Oncol. Pharm. Pract., 2010, 16, 9.
4. D. S. Shewach and R. D. Kuchta, Chem. Rev., 2009, 109, 2859.
5. D. E. Thurston, “Chemistry and Pharmacology of Anticancer Drugs”, 2007, CRC Press Taylor and Francis Group, Boca Raton.
6. Q. Hu, T. Zhou, L. Zhang, H. Li, and Y. Fang, Fresenius’ J. Anal. Chem., 2000, 368, 844.
7. Y. Sakaguchi, Y. Maehara, S. Inutsuka, I. Takahashi, M. Yoshida, Y. Emi, H. Baba, and K. Sugimachi, Cancer Chemother. Pharmacol., 1994, 33, 371.
8. S. M. Golabi and D. Nematollahi, J. Pharm. Biomed. Anal., 1992, 10, 1053.
9. O. T. Fahmy, M. A. Korany, and H. M. Maher, J. Pharm. Biomed. Anal., 2004, 34, 1099.
10. H. Lu and M. Gratzl, Anal. Chem., 1999, 71, 2821.
11. K. E. Maudens, C. P. Stove, and W. E. Lambert, J. Sep. Sci., 2008, 31, 1042.
12. C. Sottani, E. Leoni, B. Porro, B. Montagna, A. Amatu, F. Sottotetti, P. Quaretti, G. Poggi, and C. Minoia, J. Chromatogr. B, 2009, 877, 3543.
13. G. Whitaker, A. Lillquist, S. A. Pasas, R. O’Connor, F. Regan, C. E. Lunte, and M. R. Smyth, J. Sep. Sci., 2008, 31, 1828.
14. Y. Chen, R. J. Walsh, and E. A. Arriaga, Anal. Chem., 2005, 77, 2281.
15. M. Dean, A. Rzhetsky, and R. Alliksets, Genome Res., 2001, 11, 1156.
16. D. W. Shen, S. Goldenberg, I. Pastan, and M. M. Gottesman, J. Cell Physiol., 2000, 183, 108.
17. E. G. Schuetz, W. T. Beck, and J. D. Schuetz, Mol. Pharmacol., 1996, 49, 311.
18. M. M. Gottesman, T. Fojo, and S. E. Bates, Nat. Rev., 2002, 2, 49.
19. P. Borst and R. O. Elferink, Ann. Rev. Biochem., 2002, 71, 537.
20. S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, and T. Ando, Anal. Chem., 1984, 56, 111.
21. A. Dworschak and U. Pyell, J. Chromatogr. A, 1999, 848, 387.
22. K. Harada, E. Fukusaki, and A. Kobayashi, J. Biosci. Bioeng., 2006, 101, 403.
23. C. P. Chong, T. Y. Lin, C. L. Chang, Y. L. Yang, M. H. Tsai, Y. S. Yu, and M.Y. Liu, Electrophoresis, 2011, 32, 124.
24. Q. J. Gao, M. Araia, C. Leck, and A. Emmer, Anal. Chim. Acta, 2010, 662 (2), 193.
25. N. S. Hong, L. H. Shi, J. S. Jeong, I. Yang, S. K. Kim, and S. R. Park, Anal. Bioanal. Chem., 2011, 400, 2131.
26. J. Mbuna, T. Kaneta, and T. Imasaka, Electrophoresis, 2010, 31, 1396.
27. J. Mbuna, T. Kaneta, and T. Imasaka, J. Chromatogr. A, 2011, 1218, 392.
28. C. Montealegre, V. Verardo, M. L. Marina, and M. F. Caboni, Electrophoresis, 2014, 35, 779.
29. S. Bermingham, R. O’Connor, F. Regan, and G. P. McMahon, J. Sep. Sci., 2010, 33, 1571.
30. T. Hu, Q. Le, Z. Wu, and W. Wu, J. Pharm. Biomed. Anal., 2007, 43, 263.
31. C. Sottani, P. Rinaldi, E. Leoni, G. Poggi, C. Teragni, A. Delmonte, and C. Minoia, Rapid Commun. Mass Spectrom., 2008, 22, 2645.
32. K. Sakai-Kato, E. Saito, K. Ishikura, and T. Kawanishi, J. Chromatogr. B, 2010, 878, 1466.
33. S. Nussbaumer, S. Fleury-Souverain, P. Antinori, F. Sadeghipour, D. Hochstrasser, P. Bonnabry, J.-L. Veuthey, and L. Geiser, Anal. Bioanal. Chem., 2010, 398, 3033.
34. Q. Zhou and B. Chowbay, J. Pharm. Biomed. Anal., 2002, 30, 1063.
35. S. R. Urva, B. S. Shin, V. C. Yang, and J. P. Balthasar, J. Chromatogr. B, 2009, 877, 837.
36. C. M. Gilbert, R. P. McGearry, L. J. Filippich, R. L. G. Norris, and B. G. Charles, J. Chromatogr. B, 2005, 826, 273.
37. K. E. Maudens, C. P. Stove, V. F. J. Cocquyt, H. Denys, and W. E. Lambert, J. Chromatogr. B, 2009, 877, 3907.
38. N. J. Reinhold, U. R. Tjaden, H. Ith, and J. van der Greef, J. Chromatogr., 1992, 574, 327.
39. T. Pérez-Ruiz, C. Martínez-Lozano, A. Sanz, and E. Bravo, Electrophoresis, 2001, 22, 134.
40. N. Simeon, E. Chatelut, P. Canal, M. Nertz, and F. Coudrec, J. Chromatogr. A, 1999, 853, 449.
41. G. Hempel, P. Schulze-Westhoff, S. Flege, N. Laubrock, and J. Boos, Electrophoresis, 1998, 19, 2939.
42. A. R. Eder, J. S. Chen, and E. A. Arriaga, Electrophoresis, 2006, 27, 3263.
43. J. Mbuna, T. Kaneta, and T. Imasaka, Biomed. Chromatogr., 2011, 25, 1168.
44. P. T. Ricardo, Curr. Med. Chem., 2006, 13, 1859.
45. C. F. Higgins, Nature, 2007, 446, 749.
46. T. Litman, T. E. Druley, and S. E. Bates, Cell. Mol. Life Sci., 2001, 58, 931.
47. J. M. Lde Vree, E. Jacquemin, E. Sturm, D. Cresteili, P. J. Bosma, J. Aten, J.-F. Deleuze, M. Desrochers, M. Burdelski, O. Bernard, R. P. J. Oude Elferink, and M. Hadchouel, Proc. Natl. Acad. Sci. U. S. A., 1998, 95, 282.
48. S. Gergely, J. K. Paterson, J. A. Ludwig, B.-G. Catherine, and M. M. Gottesman, Nat. Rev., 2006, 5, 219.
49. A. E. van Herwaarden, W. Jonker, E. Wagenaar, R. F. Brinkhuis, J. H. Schellens, J. H. Beijnen, and A. H. Schinkel, Cancer Res., 2003, 63, 6447.
50. M. Maliepaard, G. L. Scheffer, I. F. Faneyte, M. A. van Gastelen, A. C. Pijnenborg, A. H. Schinkel, M. J. van de Vijver, R. J. Schepers, and J. H. Schellens, Cancer Res., 2001, 61, 3458.
51. N. Li, O. V. Nemirovskiy, Y. Zhang, H. Yuan, J. Mo, C. Ji, B. Zhang, T. G. Brayman, C. Lepsy, T. G. Heath, and Y. Lai, Anal. Biochem., 2008, 380, 211.
52. N. Li, J. Palandra, O. V. Nemirovskiy, and Y. Lai, Anal.
53. D. Du, H. Hu, X. Zhang, J. Chen, J. Cai, and H. Chen, *Biochemistry*, 2005, 44, 11539.
54. T. Illmer, M. Schaich, U. Oelschlagel, R. Nowak, U. Renner, B. Ziegs, S. Subat, A. Neubauer, and G. Ehninger, *Leuk. Res.*, 1999, 23, 653.
55. T. Langmann, R. Mauerer, A. Zahn, C. Moehle, M. Probst, W. Stremmel, and G. Schmitz, *Clin. Chem.*, 2003, 49, 230.
56. S. Fujimaki, T. Funato, H. Harigae, J. Fujiwara, J. Kameoka, K. Meguro, M. Kaku, and T. Sasaki, *Clin. Chem.*, 2002, 48, 811.
57. G. J. R. Zaman, M. J. Flens, M. R. van Leusden, M. de Haas, H. S. Mulder, J. Lankelma, H. M. Pinedo, R. J. Schepers, F. Baas, H. J. Broxterman, and P. Borst, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, 91, 8822.
58. N. Kartner, D. Evernden-Porelle, G. Bradley, and V. Ling, *Nature*, 1985, 316, 820.
59. V. Ferrand, F. A. M. Julian, M. M. Chauvet, M. H. Hirn, and M. J. Bourdeaux, *Cytometry*, 1996, 23, 120.
60. L. Dogan, O. Legrand, A.-M. Faussat, J.-Y. Perrot, and J.-P. Marie, *Leuk. Res.*, 2004, 28, 619.
61. L. A. Doyle, W. Yang, L. V. Abruzzo, T. Krogmann, Y. Gao, A. K. Rishi, and D. D. Ross, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, 95, 15665.
62. E. Stehfest, A. Torky, F. Glaahn, and H. Foth, *Arch. Toxicol.*, 2006, 80, 125.
63. S. Meschini, A. Calcabrini, E. Monti, D. Del Bufalo, A. Stringaro, E. Dolfini, and G. Arancia, *Int. J. Cancer*, 2000, 87, 615.
64. A. Rajagopal and S. M. Simon, *Mol. Biol. Cell*, 2003, 14, 3389.
65. N. H. Heegaard and R. T. Kennedy, *J. Chromatogr. B*, 2002, 768, 93.
66. C. C. Huang, Z. Cao, H. T. Chang, and W. Tan, *Anal. Chem.*, 2004, 76, 6973.
67. I. German, D. D. Buchanan, and R. T. Kennedy, *Anal. Chem.*, 1998, 70, 4540.
68. R. C. Tim, R. A. Kautz, and B. L. Karger, *Electrophoresis*, 2000, 21, 220.
69. B. K. Koutny, D. Schmalzing, T. A. Taylor, and M. Fuchs, *Anal. Chem.*, 1996, 68, 18.
70. Q. H. Wan and X. C. Le, *Anal. Chem.*, 2000, 72, 5583.
71. D. Schmalzing, W. Nashabeh, X. W. Yao, R. Mhatre, F. E. Regnier, N. B. Afeyan, and M. Fuchs, *Anal. Chem.*, 1995, 67, 606.
72. D. R. Driedger, R. J. LeBlanc, E. L. LeBlanc, and P. Sporns, *J. Agric. Food Chem.*, 2000, 48, 1135.
73. V. Pavski and X. C. Le, *Anal. Chem.*, 2001, 73, 6070.
74. L. Tao, C. A. Aspinwall, and R. T. Kennedy, *Electrophoresis*, 1998, 19, 403.