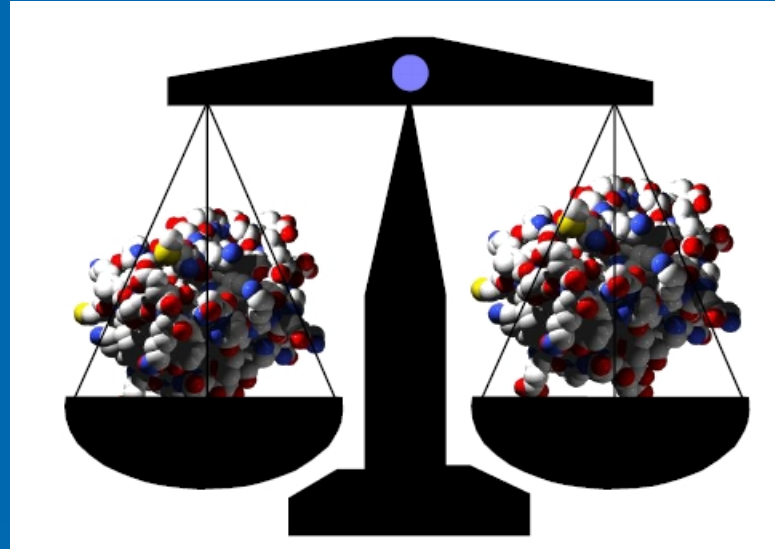


LC-MS/MS'in Klinik Laboratuvarlarda Kullanımı; İlaç, Amino Asit, Hormon ve Vitamin Analizleri



Dr. Hüseyin KAYADİBİ

Tıbbi Biyokimya Uzmanı

Adana Asker Hastanesi

KAHRAMANMARAŞ BİYOKİMYA GÜNLERİ, 07-09 KASIM 2013

SUNUM PLANI

I. Tanım, Tarihçe, Çalışma Prensibi

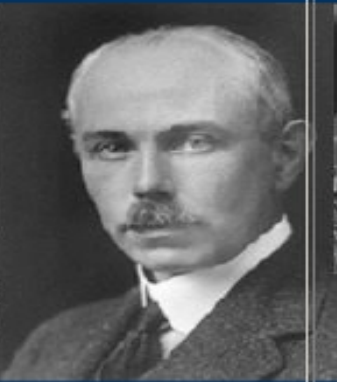
II. LC-MS/MS ile LC-MS ve Immunoassay Karşılaştırmaları

III. LC-MS/MS'in Klinik Laboratuvarlarda Kullanım Alanları

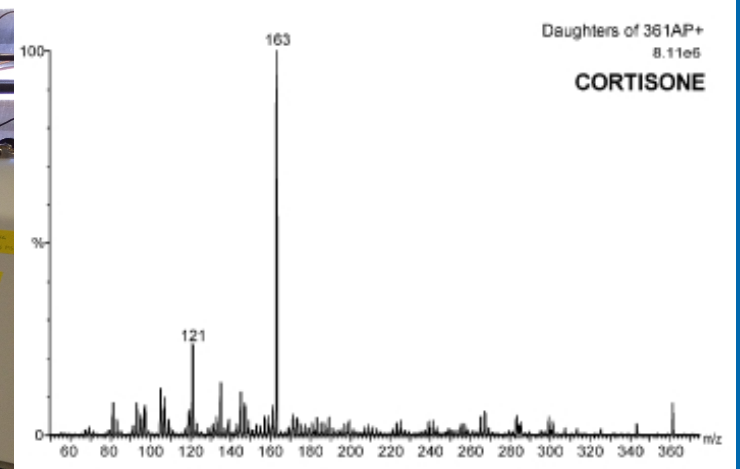
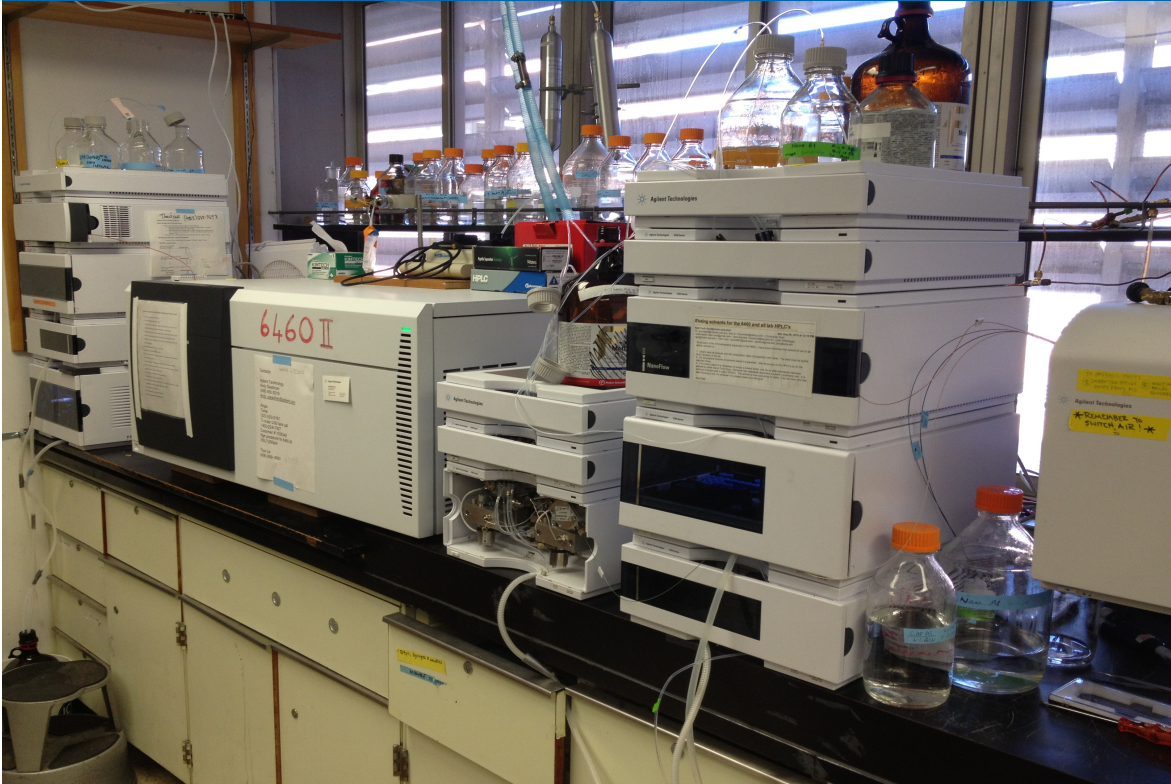
IV. Gelecek?



KÜTLE ANALİZÖRÜ NOBEL ÖDÜLLERİ

				
Joseph John Thomson 1906 Nobel Prize for Physics <i>"in recognition of the great merits of his theoretical and experimental investigations on the conduction of electricity by gases"</i>	Francis William Aston 1922 Nobel Prize for Chemistry <i>"for his discovery, by means of his mass spectrograph, of isotopes, in a large number of non-radioactive elements, and for his enunciation of the whole-number rule"</i>	Wolfgang Paul 1989 Nobel Prize for Physics <i>"for the development of the ion trap technique"</i>	John Bennet Fenn 2002 Nobel Prize for Chemistry <i>"for the development of soft desorption ionisation methods (ESI) for mass spectrometric analyses of biological macromolecules"</i>	Koichi Tanaka 2002 Nobel Prize for Chemistry <i>"for the development of soft desorption ionisation methods (MALDI) for mass spectrometric analyses of biological macromolecules"</i>

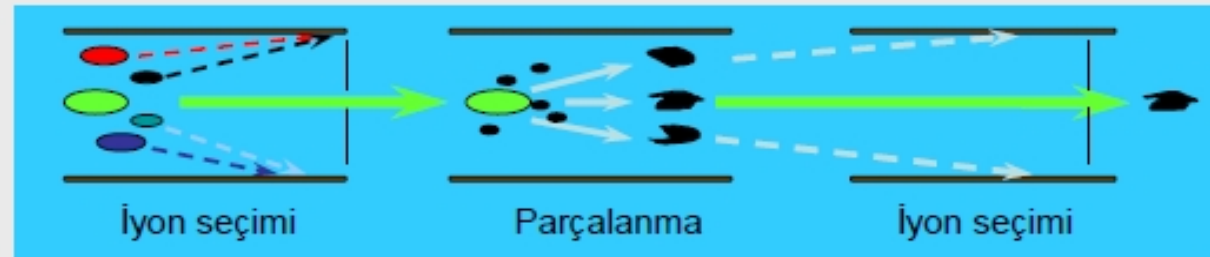
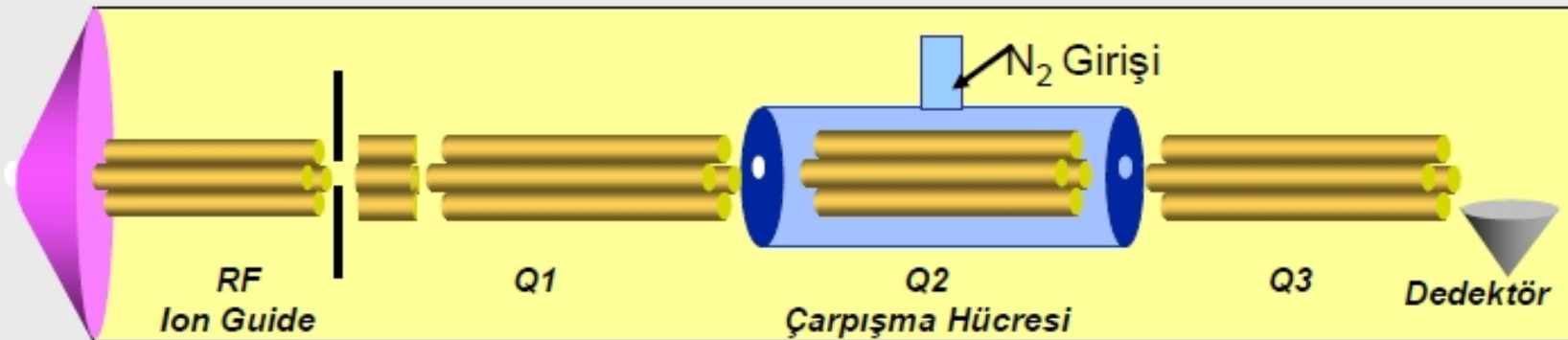
KÜTLE ANALİZÖRÜ



Gaz fazında iyonlaştırılmış molekülün kütle/yük oranını (m/z) ölçer.

1. Moleküler Ağırlık
2. Yapı
3. Miktar Tayini

Tandem MS (MS/MS) (Sıralı Kütle Analizörü)

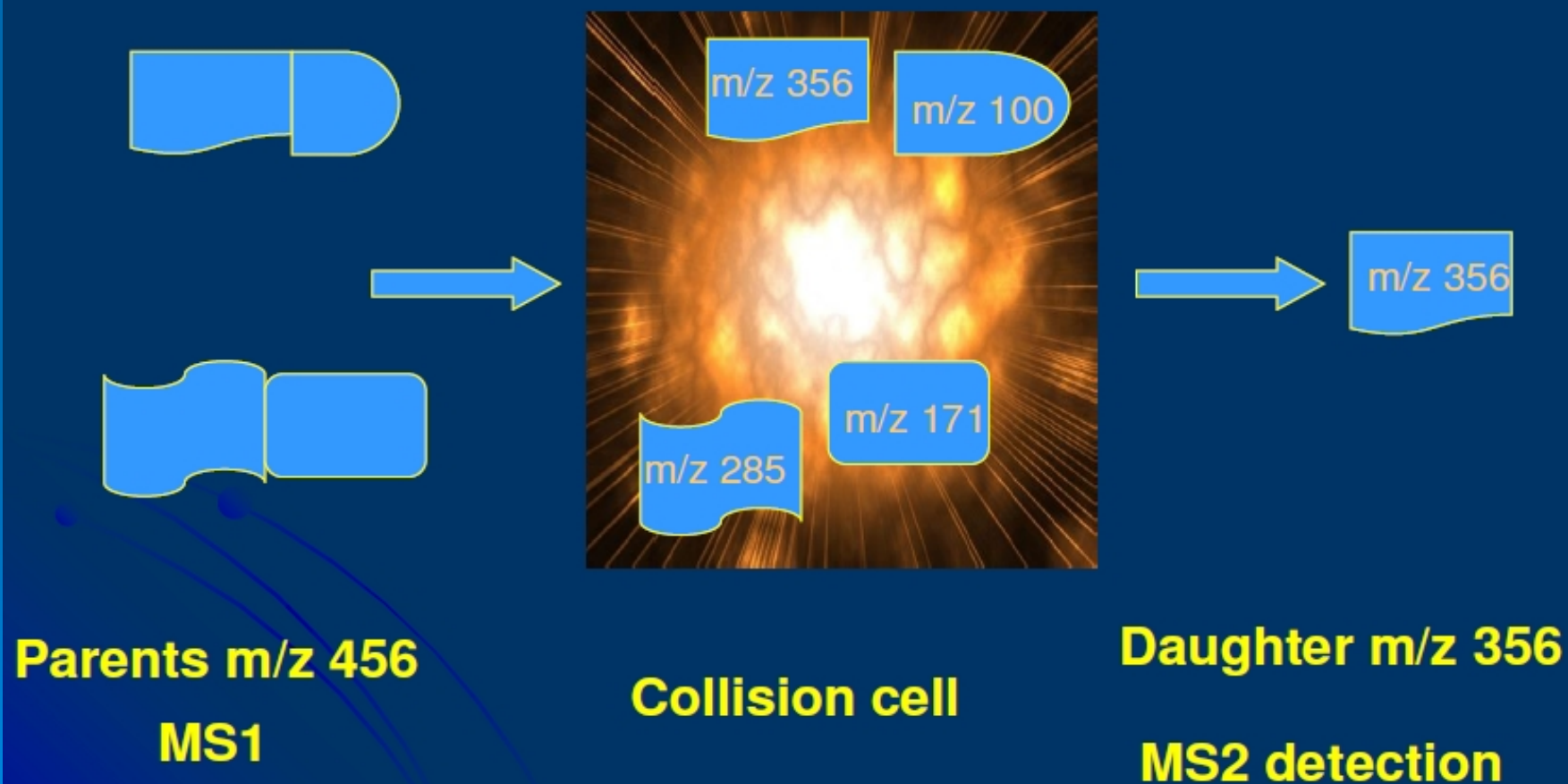


moleküler iyon/
precursor ion

ürün iyon/
product ion

LC-MS/MS PRENSIbI

Specificity through Tandem MS

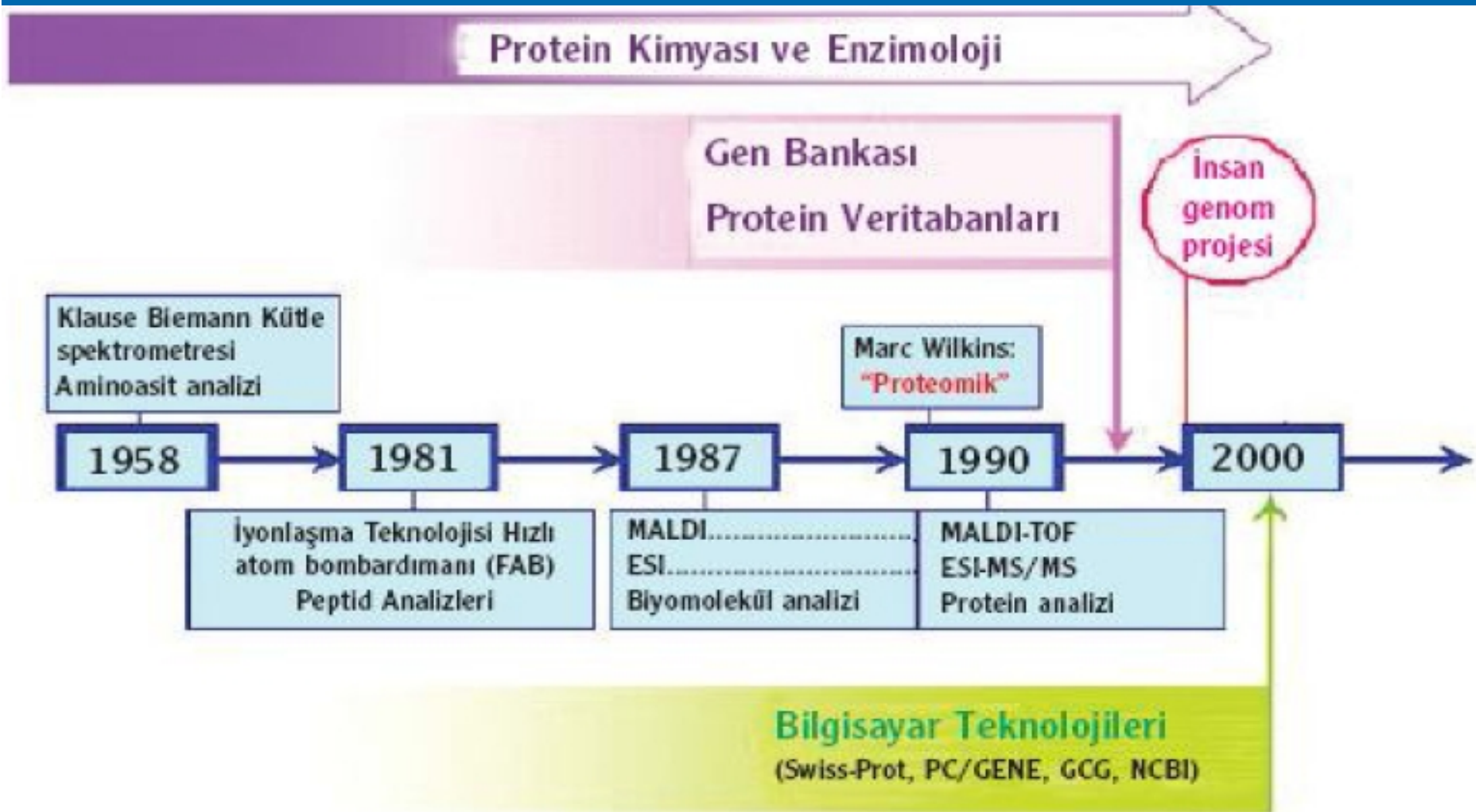


SİZCE ÖNÜMÜZDEKİ 3-5 YILDA

LC-MS/MS

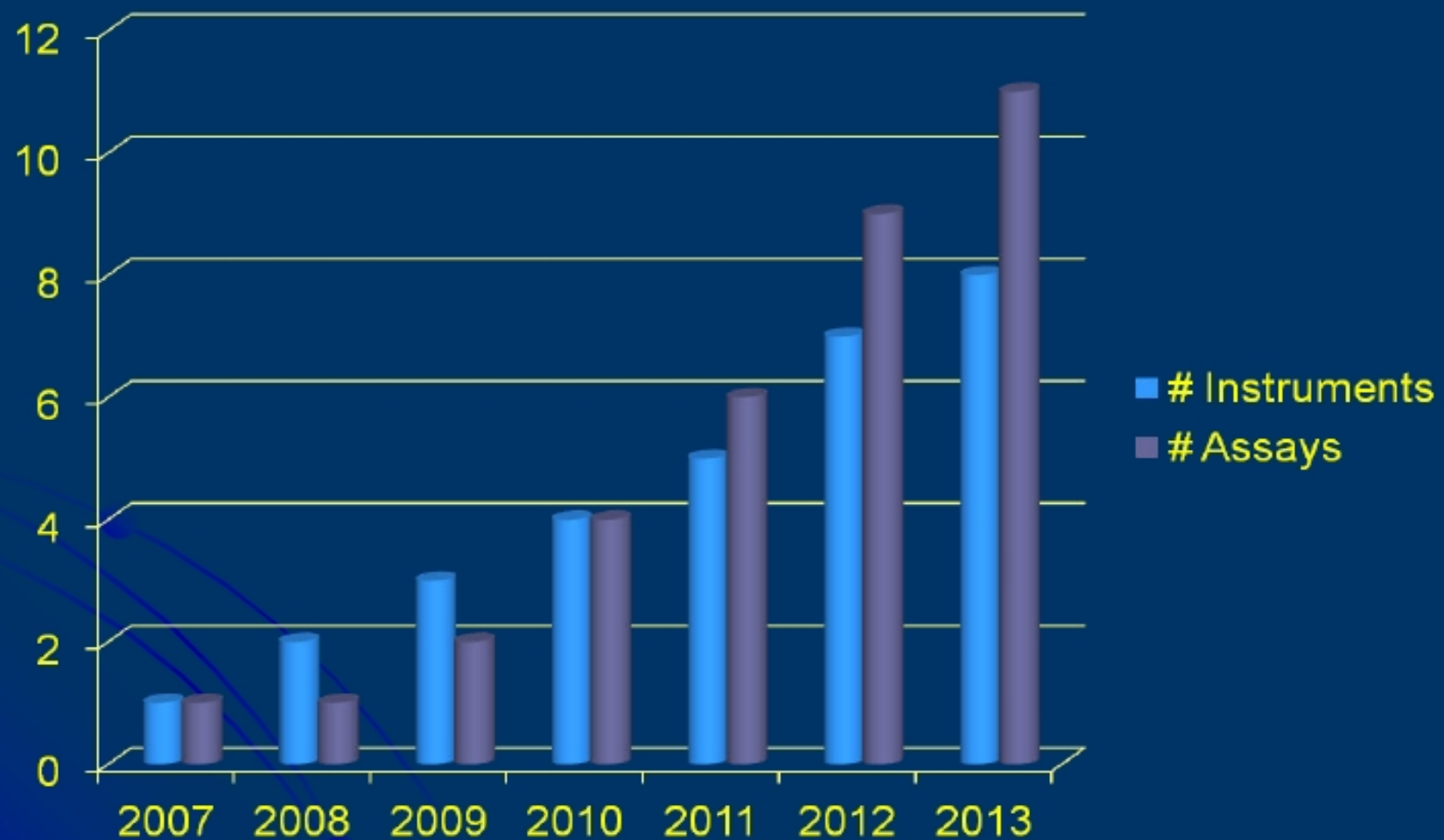
IMMUNOASSAY'IN YERİNİ ALABİLECEK Mİ?

Kütle Spektrometri Tarihsel Süreci



YAYGINLIĞI

LC-MS/MS at Cleveland Clinic



KARŞILAŞTIRMALAR

LC-MS/MS – Immunoassay

LC-MS/MS – LC-MS



GÜÇLÜ YÖNLERİ

LC-MS/MS

- Yüksek sensitivite ve spesifisite
- Az miktarda reaktif gerekliliği
- Geniş analitik ölçüm aralığı
- Aynı anda yüzlerce analitin ölçülebilmesi
- Cost-efektivite

IMMUNOASSAY

- Otomatize edilebilmeleri
- Numune ön hazırlığı olmaması
- Standardizasyon
- Düşük numune hacmi ile çalışılabilmesi

ZAYIF YÖNLERİ

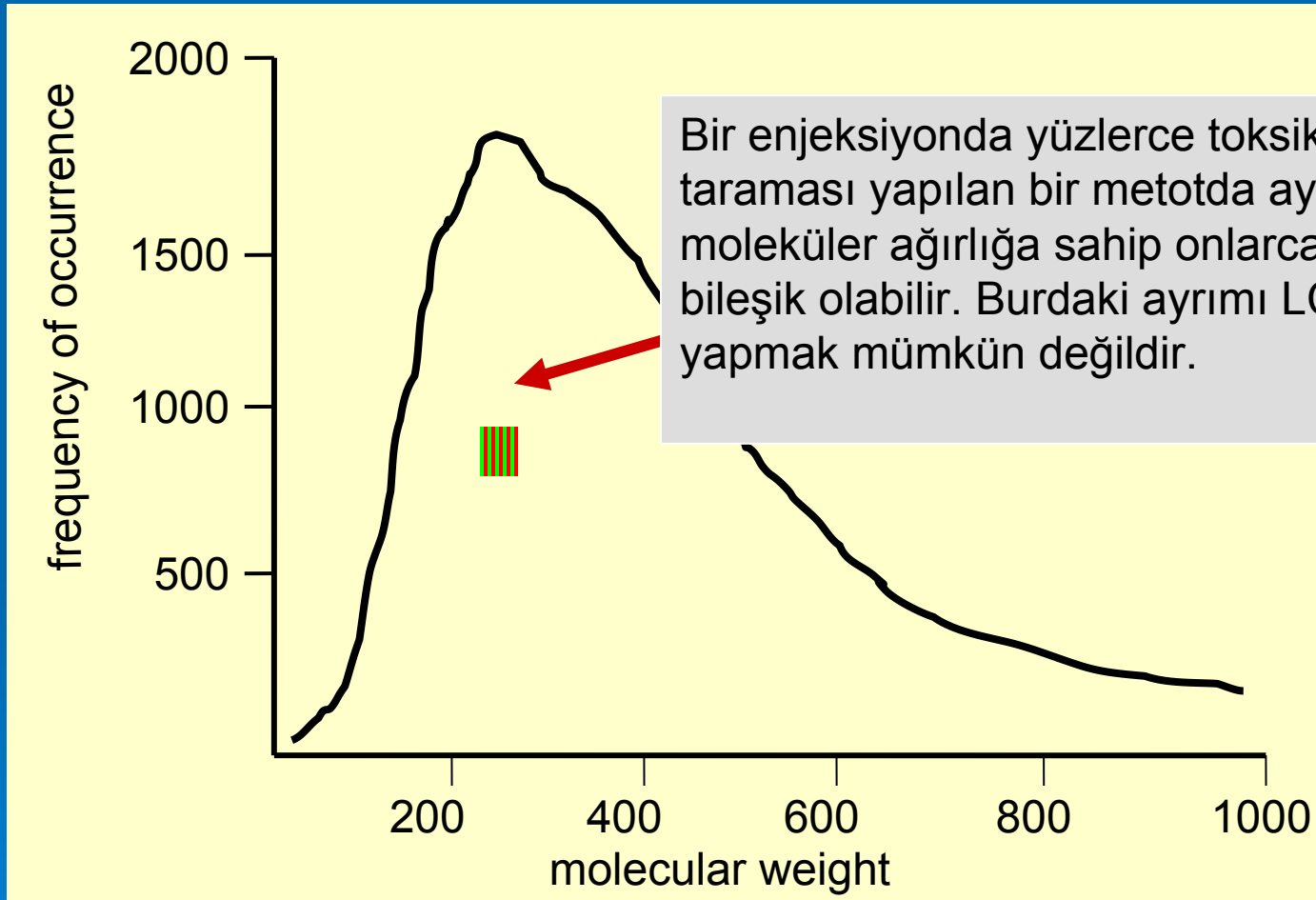
LC-MS/MS

- Manuel örnek hazırlama
- Cihaz maliyetinin yüksekliği
- Analizin kompleksliği
- Yüksek deneyimli personel gerekliliği
- Referans metod yokluğu
- Cihazın bakımı
- Otomasyon uyumsuzluğu

IMMUNOASSAY

- Düşük sensitivite
- Spesifisite problemleri, çapraz reaksiyonlar
- Yüksek reaktif maliyeti
- Dar analitik ölçüm aralığı
- Üretici firma bağımlılığı
- Her bir testin ayrı ayrı injekte edilmesi

NEDEN LC-MS YERİNE LC-MS/MS TERCİH EDİLİR?



LC-MS/MS KULLANIM ALANLARI



TÜM KLİNİK BİYOKİMYA ANALİTLERİNİ
LC-MS/MS İLE ÖLÇEBİLİR MİYİZ?

EVET, AMA BİR ŞARTLA

GAZ FAZINDA

İYONİZASYON

Low levels of circulating estrogen sensitize PTEN-null endometrial tumors to poly(ADP-ribose) polymerase (PARP) inhibition in vivo

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Quantification of rifampicin in human plasma and cerebrospinal fluid by a highly sensitive and rapid liquid chromatographic–tandem mass spectrometric method

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Cerebrospinal fluid

Validation

Quantitative assay

ABSTRACT

A highly sensitive and rapid liquid chromatography tandem mass spectrometry (LC–MS/MS) method has been developed to measure the levels of the antitubercular drug rifampicin (RIF) in human plasma and cerebrospinal fluid (CSF). The analyte and internal standard (IS) were isolated from plasma and CSF by a simple organic solvent based precipitation of proteins followed by centrifugation. Detection was carried out by electrospray positive ionization mass spectrometry in the multiple-reaction monitoring (MRM) mode. The assay was linear in the concentration range 25–6400 ng/mL with intra- and inter-day precision of <7% and <8%, respectively. The validated method was applied to the study of RIF pharmacokinetics in human CSF and plasma over 25 h period after a 10 mg/kg oral dose.

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Importance of highly selective LC–MS/MS analysis for the accurate quantification of tamoxifen and its metabolites: focus on endoxifen and 4-hydroxytamoxifen

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J. H. M. Schellens · J. H. Beijnen

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Abstract The antiestrogenic effect of tamoxifen is mainly attributable to the active metabolites endoxifen and 4-hydroxytamoxifen. This effect is assumed to be concentration-dependent and therefore quantitative analysis of tamoxifen and metabolites for clinical studies and therapeutic drug monitoring is increasing. We investigated the large discrepancies in reported mean endoxifen and 4-hydroxytamoxifen concentrations. Two published LC–MS/MS methods are used to analyse a set of 75 serum samples from patients treated with tamoxifen. The method from Teunissen et al. (J Chrom B, 879:1677–1685, 2011) separates endoxifen and 4-hydroxytamoxifen from other

tamoxifen metabolites with similar masses and fragmentation patterns. The second method, published by Gjerdet et al. (J Chrom A, 1082:6–14, 2005) however lacks selectivity, resulting in a factor 2–3 overestimation of the endoxifen and 4-hydroxytamoxifen levels, respectively. We emphasize the use of highly selective LC–MS/MS methods for the quantification of tamoxifen and its metabolites in biological samples.

Keywords Tamoxifen · Endoxifen · 4-Hydroxytamoxifen · Metabolite levels · LC–MS/MS analysis

A Simple Liquid Chromatography–Tandem Mass Spectrometry Method for Determination of Plasma Fentanyl Concentration in Rats and Patients with Cancer Pain

Tatsuya Hisada,^{a,b} Miki Katoh,^a Kotaro Hitoshi,^a Yuya Kondo,^a Miho Fujioka,^a Yukio Toyama,^{b,c} Hideaki Ieda,^d Saori Gocho,^e and Masayuki Nadai^{*,a}

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Application of LC-MS/MS analysis of plasma amino acids profiles in children with autism

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J Chromatogr B Analyt Technol Biomed Life Sci. 2011 March 1; 879(7-8): 467–474. doi:10.1016/j.jchromb.2011.01.006.

Simultaneous Bioanalysis of L-Arginine, L-Citrulline, and Dimethylarginines by LC-MS/MS

Soyoung Shin^a, Sun-Mi Fung^a, Srinidi Mohan^a, and Ho-Leung Fung^{a,*}

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J Chromatogr B Analyt Technol Biomed Life Sci. 2011 August 15; 879(24): 2389–2396. doi:10.1016/j.jchromb.2011.06.034.

A LC–MS/MS method for the specific, sensitive, and simultaneous quantification of 5-aminolevulinic acid and porphobilinogen

Jinglan Zhang, Makiko Yasuda, Robert J. Desnick, Manisha Balwani, David Bishop, and Chunli Yu*

Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, Fifth Avenue at 100th Street, New York, NY 10029, USA

Anal Biochem. 2012 June 15; 425(2): 145–150. doi:10.1016/j.ab.2012.03.007.

An LC-MS/MS Assay for Detection and Quantification of the Dipeptide Gly-Gln in Rat Brain

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^aDivision of Pharmaceutical Sciences, School of Pharmacy, University of Missouri-Kansas City, 2464 Charlotte Street, Kansas City, Missouri 64108, USA.

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Anal Bioanal Chem. 2010 July ; 397(5): 1831–1839. doi:10.1007/s00216-010-3705-9.

ANALYSIS OF THYROID HORMONES IN SERUM BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

Dongli Wang and Heather M. Stapleton*

Nicholas School of the Environment, Duke University, Durham, NC 27708, USA

Clin Chem. 2009 June ; 55(6): 1061–1066. doi:10.1373/clinchem.2007.100008.

Steroid Hormone Analysis by Tandem Mass Spectrometry

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²Department of Pharmacology and General Clinical Research Center, Georgetown University, Washington, DC

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⁴Department of Physiology and Biophysics, Georgetown University, Washington, DC

Endocrine Journal 2009, 56 (9), 1083-1093

Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) Assay for Simultaneous Measurement of Salivary Testosterone and Cortisol in Healthy Men for Utilization in the Diagnosis of Late-onset Hypogonadism in Males

FUTOSHI MATSUI*, EITETSU KOH*, KENROU YAMAMOTO*, KAZUHIRO SUGIMOTO*, HO-SU SIN*, YUJI MAEDA*, SEIJIRO HONMA** AND MIKIO NAMIKI*

**Department of Integrative Cancer Therapy and Urology, Kanazawa University Graduate School of Medical Science, Kanazawa, Ishikawa 920-8641, Japan*

***ASKA Medical Co. Ltd., Kawasaki, Kanagawa 213-8522, Japan*

Quantification of $1\alpha,25$ -Dihydroxy Vitamin D by Immunoextraction and Liquid Chromatography–Tandem Mass Spectrometry

Frederick G. Strathmann,¹ Thomas J. Laha,¹ and Andrew N. Hoofnagle^{1,2*}

C3 epimeri; infant

A decorative graphic consisting of several concentric circles of varying sizes, rendered in a lighter blue shade than the background, located in the bottom right corner of the slide.

High-Sensitivity Tandem Mass Spectrometry Assay for Serum Estrone and Estradiol

*Mark M. Kushnir, MS,^{1,3} Alan L. Rockwood, PhD,^{1,2} Jonas Bergquist, PhD,³
Marina Varshavsky, MS,¹ William L. Roberts, MD, PhD,^{1,2} Bingfang Yue, PhD,¹ Ashley M. Bunker,¹
and A. Wayne Meikle, MD^{1,2,4}*

Key Words: Estradiol; Estrone; Estrogens; Reference intervals; Tanner stage; Mass spectrometry

DOI: 10.1309/LC03BHQ5XJPJYEKG

Rapid 2nd-Tier Test for Measurement of 3-OH-Propionic and Methylmalonic Acids on Dried Blood Spots: Reducing the False-Positive Rate for Propionylcarnitine during Expanded Newborn Screening by Liquid Chromatography–Tandem Mass Spectrometry

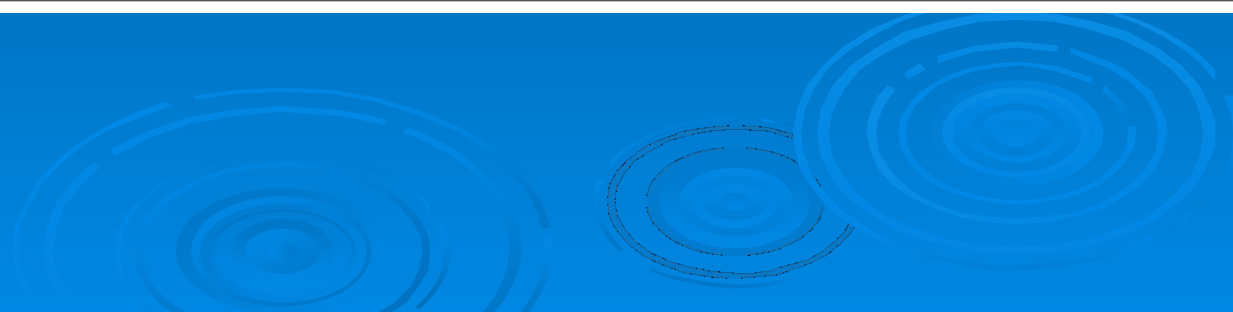
GIANCARLO LA MARCA,^{1*} SABRINA MALVAGIA,¹ ELISABETTA PASQUINI,¹ MARZIA INNOCENTI,²
MARIA ALICE DONATI,¹ and ENRICO ZAMMARCHI¹

Clinical Chemistry 50:3
621–625 (2004)

Endocrinology and
Metabolism

Improved Specificity of Newborn Screening for Congenital Adrenal Hyperplasia by Second-Tier Steroid Profiling Using Tandem Mass Spectrometry

JEAN M. LACEY,¹ CARLA Z. MINUTTI,^{1,2} MARK J. MAGERA,¹ ANGELA L. TAUSCHER,¹
BRUNO CASETTA,⁶ MARK McCANN,⁷ JAMES LYMP,⁵ SI HOUN HAHN,^{1,3,4} PIERO RINALDO,^{1,3,4}
and DIETRICH MATERN^{1,3,4*}



Second-Tier Test for Quantification of Alloisoleucine and Branched-Chain Amino Acids in Dried Blood Spots to Improve Newborn Screening for Maple Syrup Urine Disease (MSUD)

Devin Oglesbee,^{1,2} Karen A. Sanders,¹ Jean M. Lacey,¹ Mark J. Magera,¹ Bruno Casetta,⁴ Kevin A. Strauss,⁵
Silvia Tortorelli,^{1,2} Piero Rinaldo,^{1,2,3} and Dietrich Matern^{1,2,3*}



J Mass Spectrom. 2012 March ; 47(3): 277–285. doi:10.1002/jms.2051.

Quantitation of Melatonin and N-acetylserotonin in Human Plasma by Nanoflow LC-MS/MS and Electrospray LC-MS/MS

Melissa D. Carter^{*}, M. Wade Calcutt^{*}, Beth A. Malow[‡], Kristie L. Rose^{*}, and David L. Hachey^{*,†,§}

^{*}Vanderbilt University, Department of Biochemistry, Nashville, TN 37235, USA

[†]Vanderbilt University, Department of Pharmacology, Nashville, TN 37232, USA

[‡]Vanderbilt University, Department of Neurology, Nashville, TN 37232, USA



Plasma Renin Activity by LC-MS/MS:
Development of a Prototypical Clinical Assay Reveals a
Subpopulation of Human Plasma Samples with Substantial
Peptidase Activity

Cory E. Bystrom,¹ Wael Salameh,¹ Richard Reitz,¹ and Nigel J. Clarke^{1*}

LC-MS/MS Kullanım Alanları

Klinik Uygulamalar

- Yeni doğan taramaları; aminoasitler, yağ asidi oksidasyon defektleri, organik asidemi ve asidüri
- Yeni doğan hastalıklarının doğrulanması; tirozinemi, propionik asidemi, MMA, MSUD, lizozomal depo hastalıkları, FKÜ
- Steroid hormonlar; E1, E2, progesteron, testesteron, kortizol (serum, idrar ve tükürük), aldosteron, androstenedion, kortizol, kortizon, 11-deoksikortizol, 21-deoksikortizol, 17-OH-P, DHEA, DHEAS)
- D Vitamini; D2, D3, 3-epi izomerleri
- Vitaminler
- İnsülin, T3, T4
- Hemoglobin analizleri
- İlaçlar
- Karbohidratlar

LC-MS/MS Kullanım Alanları

Biyoteknoloji

- Protein, peptid ve oligonükleotidler

Adli Tıp

Toksikoloji

Farmasötik Uygulamalar

- Farmakokinetik, ilaç metabolizması, ilaç geliştirme



SABRINIZ İÇİN TEŞEKKÜR EDERİM

