The coupling of HPTLC with DART mass spectrometry

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DART = Direct Analysis in Real Time

(R.B. Cody et al., 2005)

DART is an "**open-air**" **ionization source** for mass spectrometry, enabling the ultrafast analysis of solid or liquid samples in the gaseous stream **WITHOUT a sample preparation**

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DART mass spectrometry and its applications in chemical analysis

E S Chernetsova, G E Morlock, I A Revelsky

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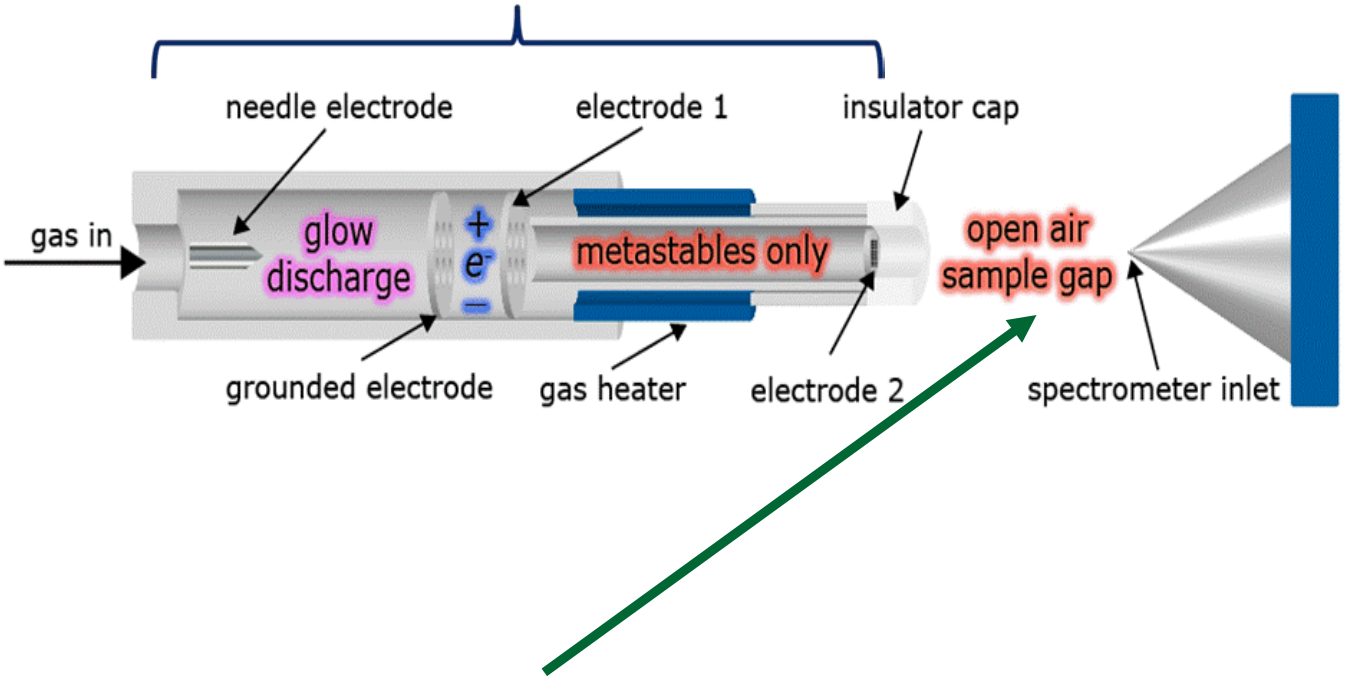
Abstract. Published data on the fundamental and applied aspects of DART mass spectrometry are surveyed. The DART ionization principles and the key parameters affecting the analytical characteristics of the method and the mass spectra of the determined compounds are considered. The advantages and drawbacks of DART mass spectrometry are discussed and the existing and prospective applications are outlined. The bibliography includes 120 references.

ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) techniques. Presently, DART-MS studies are rapidly progressing and the number of publications on the subject and corresponding analytical applications increases continuously.

DART mass spectrometry allows one to carry out a fast non-contact analysis of various samples. Most often, solid samples are introduced into the ionization region with a

Direct Analysis in Real Time mass spectrometry (DART-MS)

DART: „open-air“ ionisation source



Volatilization / Desorption / Ionization

reproduced from www.ionsense.com

Coupling TLC/HPTLC with DART-MS

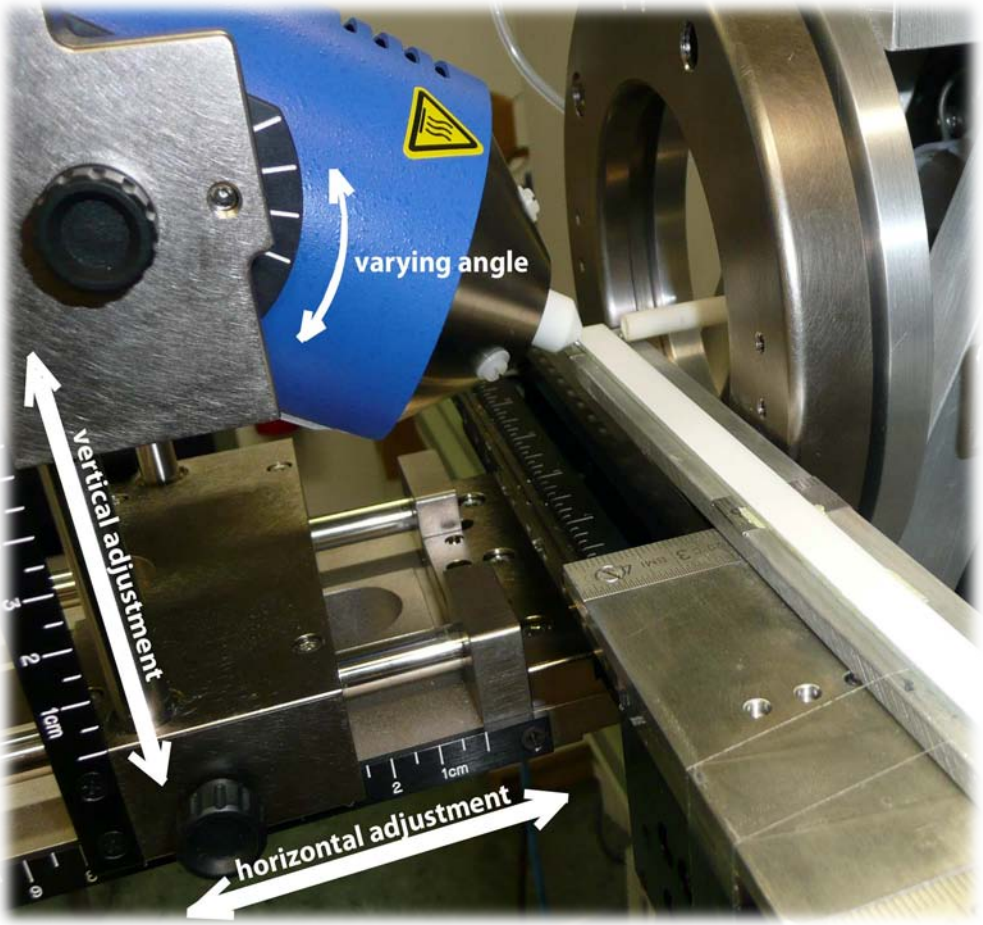
2006-2010

- **Horizontal** gas supply
- **Manual** plate introduction
- **Low reproducibility!**

Coupling TLC/HPTLC with DART-MS

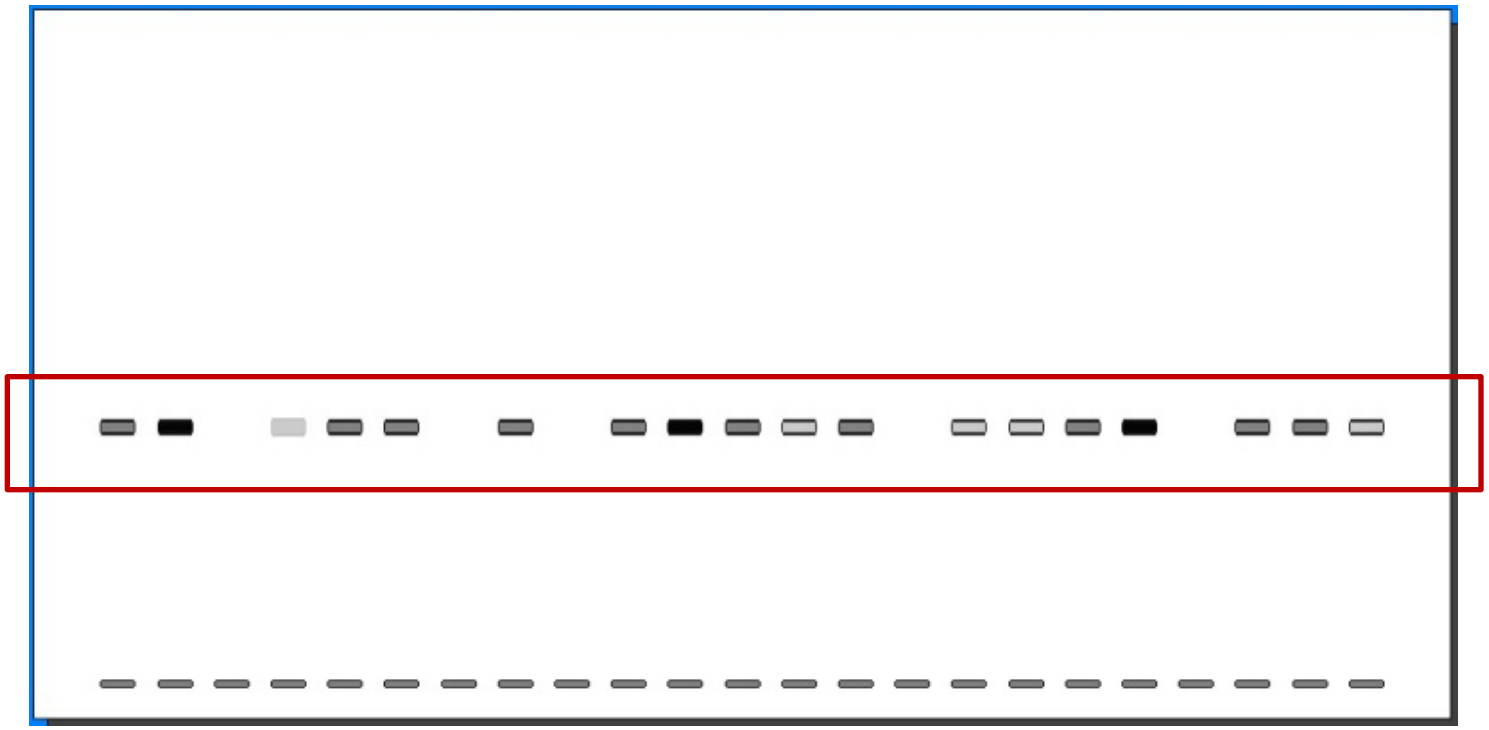
2011

- ✓ Desorption **at an angle**
- ✓ Ability for **scanning** across the TLC plates
- ✓ **Positioning** *at the focus of the gas beam* is critical



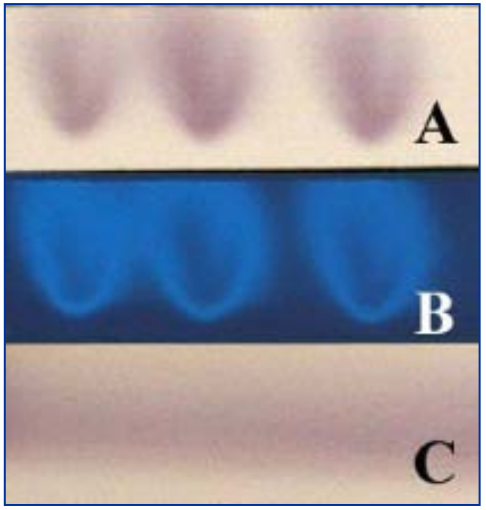
Coupling TLC/HPTLC with DART-MS

Attractive:
scanning in the ‚substance window'



Coupling TLC/HPTLC with DART-MS

visualisation *of the gas beam*
based on colour change during a chemical reaction

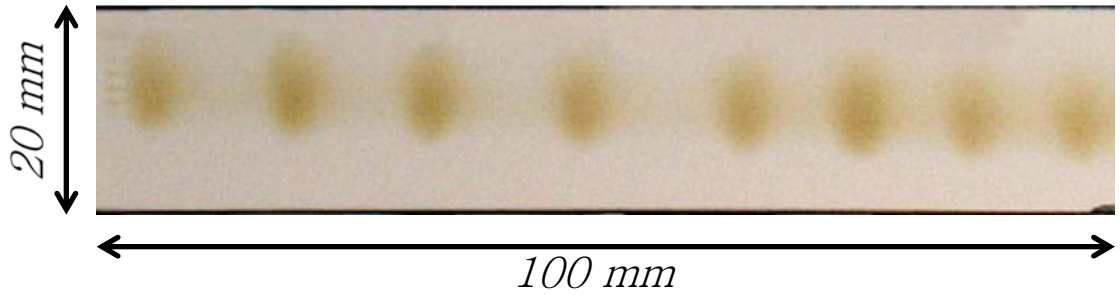


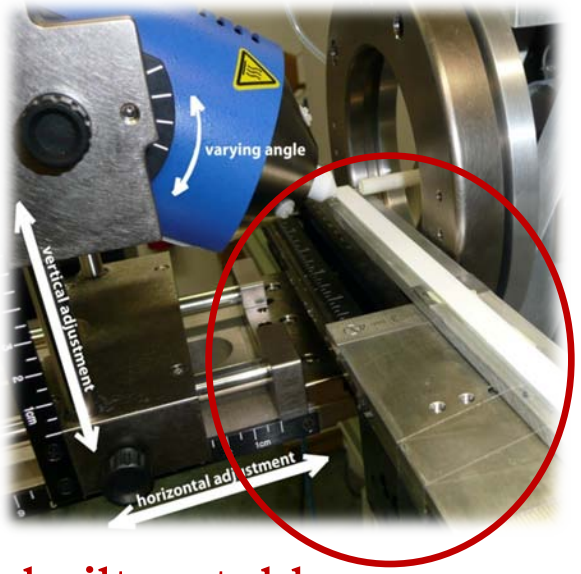
Visualisation for the HPTLC plate, treated with sugar solution + β-naphthol reagent

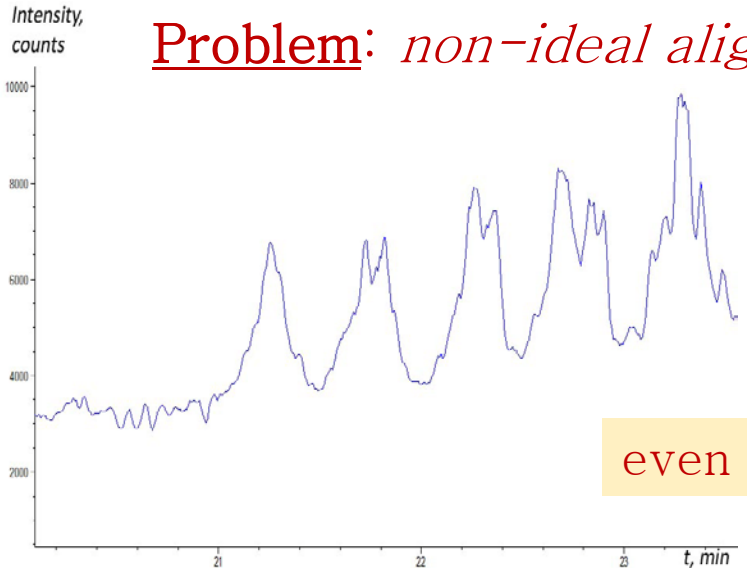
for ***optimal positioning*** *of a TLC plate in DART-MS*

E.S. Chernetsova et al. Some new features of Direct Analysis in Real Time mass spectrometry utilizing the *desorption at an angle* option // Rapid Commun. Mass Spectrom., 2011. *In press*.

Coupling TLC/HPTLC with DART-MS



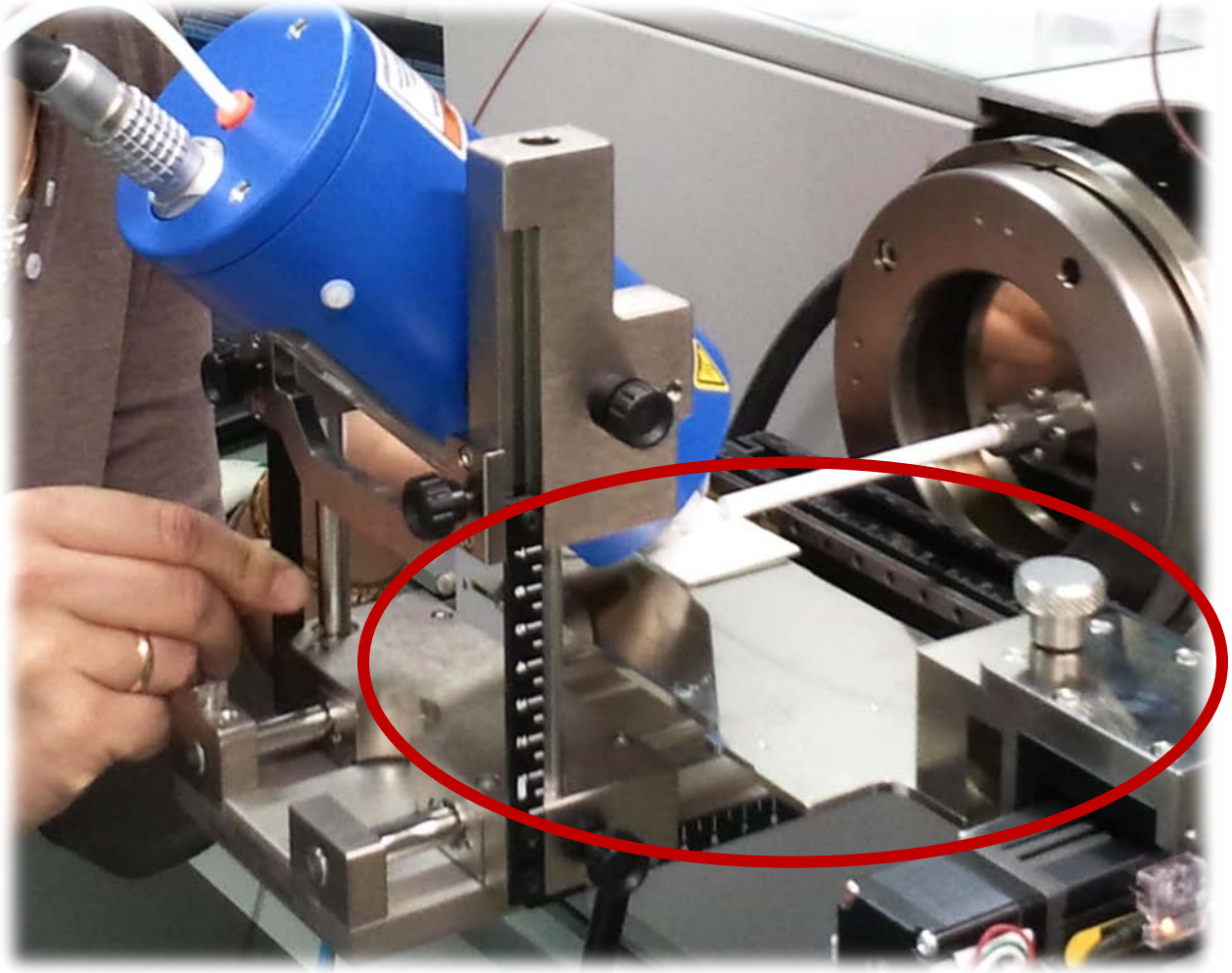




Problem: *non-ideal alignment* of the **home-built *x*-table**

even the **non-alignment of *1 mm*** is critical

Coupling TLC/HPTLC with DART-MS

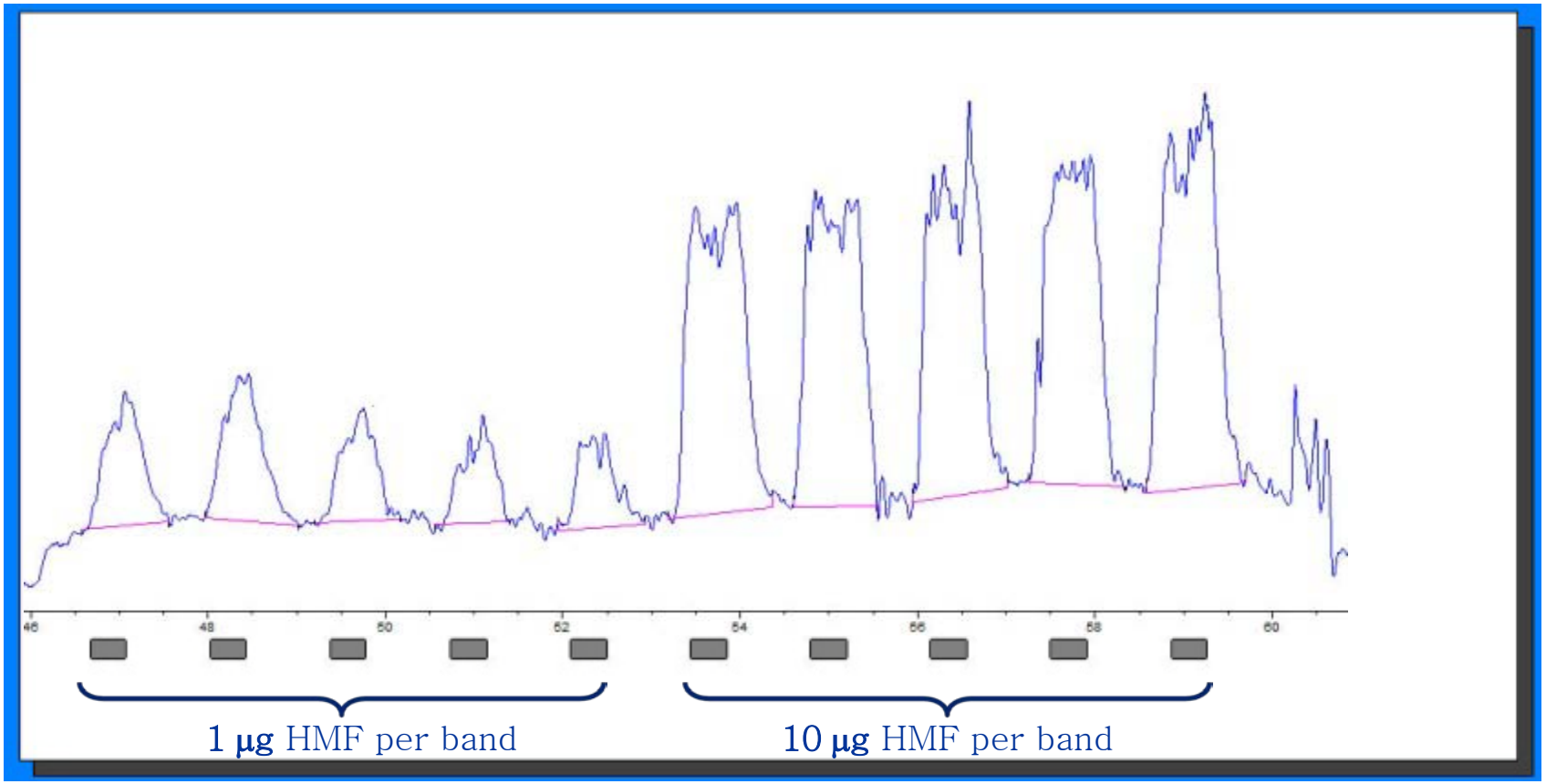


AVAILABLE NOW: *x*-*y*-*z*-table for DART

Coupling TLC/HPTLC with DART-MS

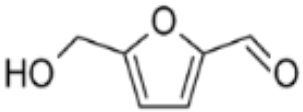
AVAILABLE NOW: *x-y-z*-table for DART

Coupling TLC/HPTLC with DART-MS



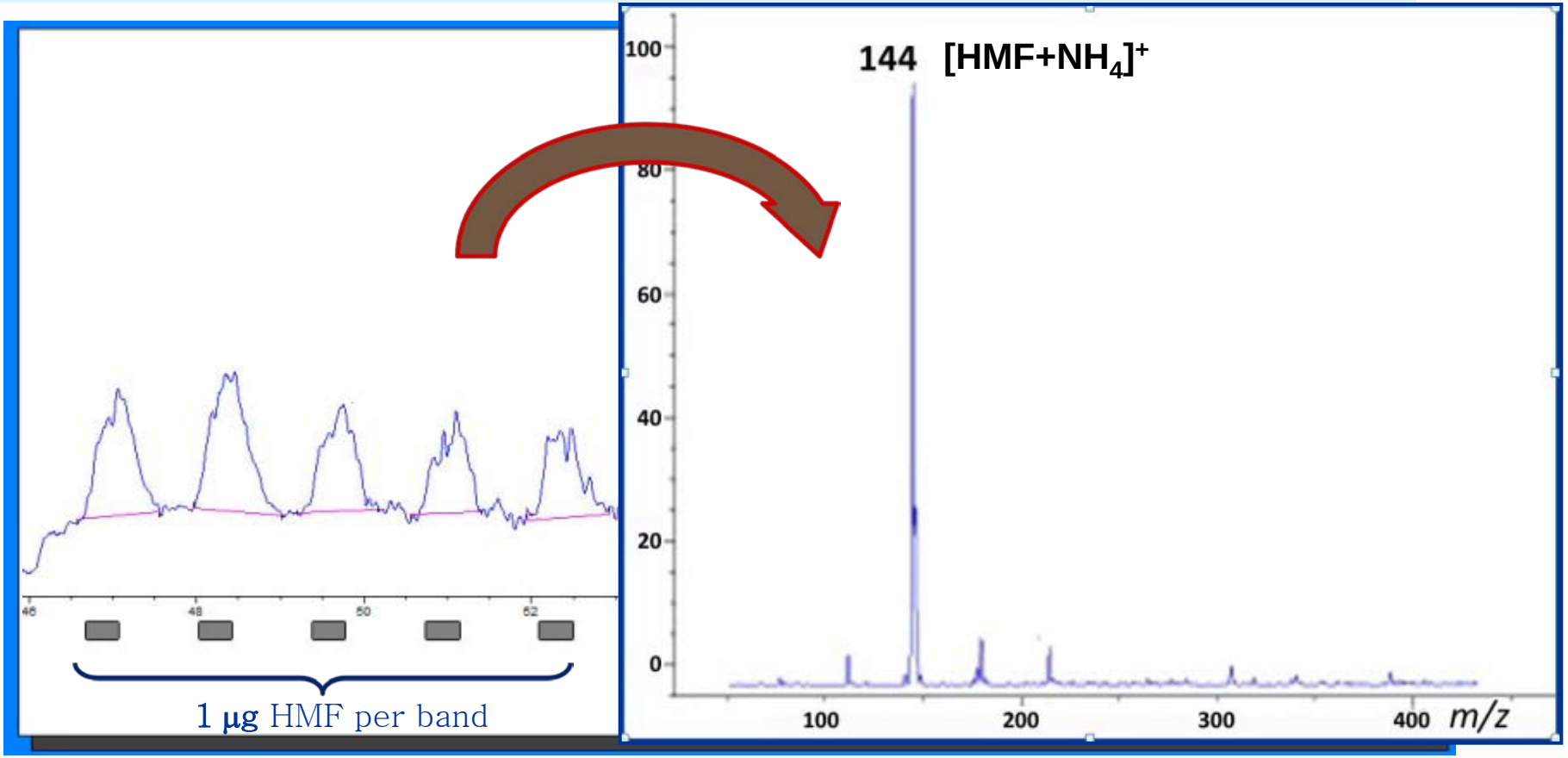
horizontal scanning by *DART-MS*

RSD of 15% and better



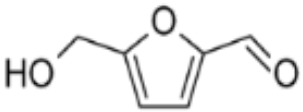
HMF – 5-hydroxymethylfurfural

Coupling TLC/HPTLC with DART-MS



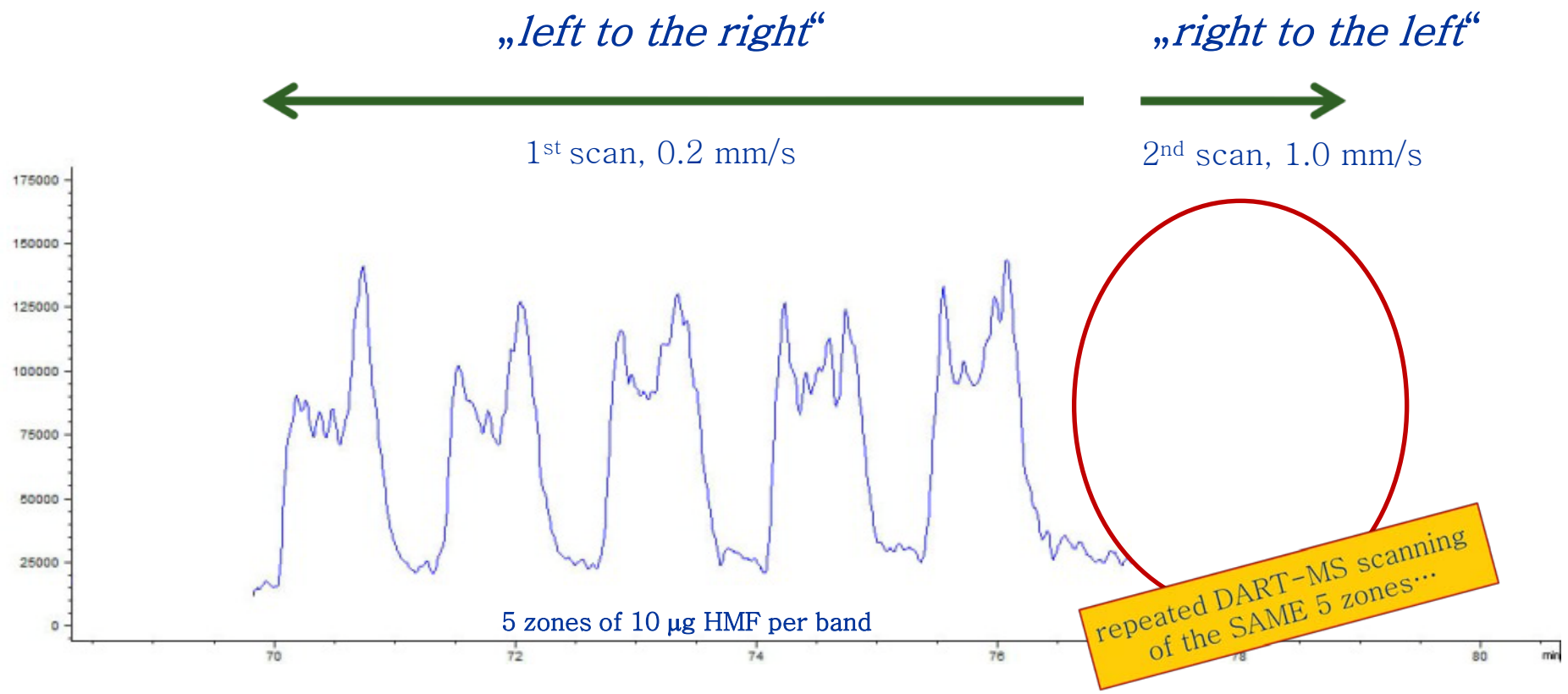
horizontal scanning by DART-MS

RSD of 15% and better



HMF – 5-hydroxymethylfurfural

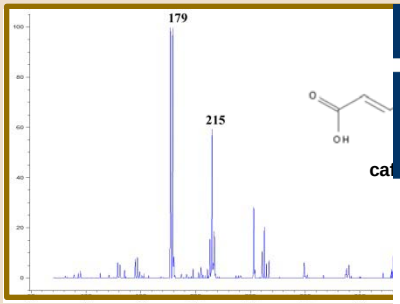
Coupling TLC/HPTLC with DART-MS

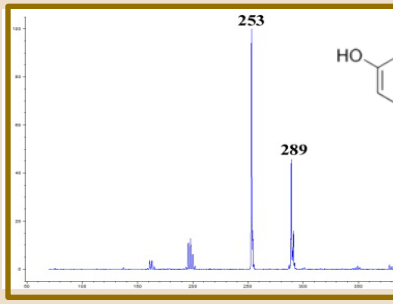


Identification of flavonoids in propolis

HPTLC-ESI-MS:

Elution-Head-based HPTLC-MS





poster # 8j

New HPTLC method for analysis of flavonoids and phenolic compounds in propolis

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[2]Apicultural State Institute, Stuttgart, Germany
*In cooperation with WALA Heilmittel GmbH

Introduction

Propolis is a complex product of bees, which they collect from resinated buds of different plants (Fig. 1) and then use as a glue building their hive. It is often used in medicinal and cosmetic preparations due to its valuable properties. In propolis flavonoids and other phenolic compounds are treasured components of primary interest, as they show various interesting biological activities, such as improvement of blood pressure, removal of oxygen radical, anti-cancer, antibacterial and antibiotic action. In our opinion, planar chromatography can become a method of choice for characterization of samples rich in such compounds as for reliable identification of sample components, planar chromatography can now be coupled online and reliable to mass spectrometry. Therefor new HPTLC-MS hyphenations were used via the **Direct Analysis in Real Time DART (DART)** ion source working under ambient conditions and the **TLC-MS Interface** allowing to couple planar chromatography to such a widely used ionization source for mass spectrometry as electrospray ionization (ESI). The intention was to establish a new method suited for screening of the still unknown chemical profile of German propolis sorts, for differentiation between different types of propolis and for assignment of the plant origin of the propolis samples.

Results and discussion

Method development

The separation was performed on HPTLC plates silica gel 60. After systematic mobile phase optimization, the best separation conditions were obtained with a mixture of *n*-hexane, ethyl acetate and acetic acid as mobile phase and acidic conditioning of the plate. The separation of up to 20 samples last less than 30 min (migration distance 60 mm). Detection was performed in the multi-wavelength scan before and after derivatization with Neu´s reagent and enhancement of various zones by polyethylene glycol.

Analysis of different propolis samples

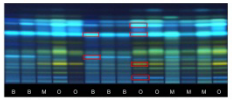

Fig. 2 HPTLC separation of the extracts of **German** propolis, which shows the characteristic pattern of markers for 2 main types of propolis (highlighted with red), O: Orange type, B: Blue type, M: mixture of both types

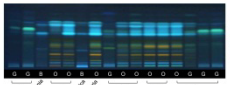

Fig. 3 HPTLC separation of the extracts of propolis from **other countries**, which also shows the Green type (G) pattern for propolis beside the Orange type (O) and Blue type (B) samples

A screening of more than **100 German** propolis samples of different locations and years revealed two main types of propolis: one with a pattern dominated by the color blue and another type also containing orange marker compounds (Fig. 2). Mixtures of both types are possible. Propolis samples from other countries also show the Orange type and a „Blue" type which slightly differs from the German one. Additionally, most of the Brasilian propolis samples showed a different pattern with a bright green zone (Fig. 3) that could not be detected in any German or even European propolis samples.

Analysis of plant extracts

For assignment of the plant origin of German propolis, HPTLC separations of plant extracts were performed (Fig. 4). Black popolar extract showed a very similar pattern to the Orange type samples and is most likely to represent the origin of this type. Further investigations are needed for indication of the origin of the Blue type samples.

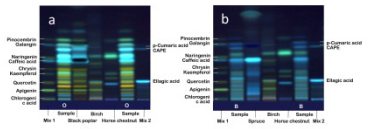

Fig. 4 Comparison of the HPTLC pattern of the **Orange** (O; 4a) and **Blue** type samples (B; 4b) with various plant extracts and reference standard mixtures of flavonoids and phenolic compounds

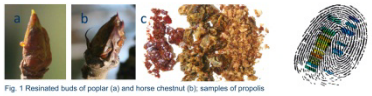

Fig. 1 Resinated buds of poplar (a) and horse chestnut (b); samples of propolis

Couplings of HPTLC with mass spectrometry

Coupling of HPTLC with mass spectrometry was performed on non-derivatized plates using the DART ion source with the *desorption at an angle* option and an x/y/z-motor stage (Fig. 5, left) or the TLC-MS-Interface coupled to an ESI ion source (Fig. 5, right).

For HPTLC-DART-MS analysis, the propolis extracts were applied on the HPTLC plate (track distance 9 mm, band length 5.5 mm), if necessary, cut to strips. After separation, selected extracts/tracks were recorded by scanning in the total ion current chromatogram (TIC) mode at the lowest possible speed of 0.2 mm/s. The DART temperature was set to 300 ˚C to support analyte desorption from the surface. Though using this high temperature, the desorption was incomplete (only on the upper sur-face), which generally allows to subject the same HPTLC extract to further MS analyses, *e.g.* with the TLC-MS Interface. HPTLC-DART-MS allowed to locate the phenolic markers via the extracted ion chromatograms (EIC) at their characteristic *m/z* values (Fig. 5, left down).

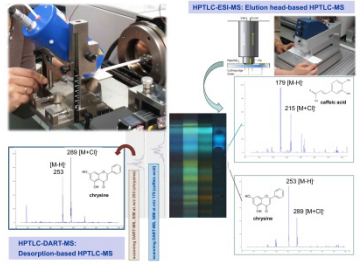

Fig. 5 Compound identity confirmation by HPTLC-DART-MS (direct scanning of the whole extract or a region, desorption-based; left) and HPTLC-ESI-MS (targeted access to zones of interest, elution-based; right)

For HPTLC-ESI-MS analysis (Fig. 5, right), the analyte zones were marked with a soft pencil, and then the online elution of a zone into the ESI-MS was performed. The eluted zone can directly be subjected to ESI-MS, or for co-eluting zones, can be directed via an integrated HPLC column (HPTLC-HPLC-ESI-MS). Also, using the same extraction interface, it was possible to collect the eluted zones into separate vials and subject them *offline* for further analyses, *e.g.*, using ion trap or Orbitrap MS analyzers. Such *offline* approaches were used for identification of unknown phenolic markers of propolis extracts after their HPTLC separation.

Conclusions

Planar chromatography coupled with MS for screening of complex extracts as well as for quantification and identification of their marker compounds is a very useful, efficient technique for today's analysts!

Thanks to Dr. Dogan (Merck) for support regarding plate material, to R. Rolli and Dr. Natsias (CAMAG) concerning quantitative instrumental equipment, and to Professor Dr. Schwack (University of Hohenheim) for the excellent conditions at the Institute. This work was financially supported within the joint DAAD-Rosobrazovanie program "*Mikhail Lomonosov*".

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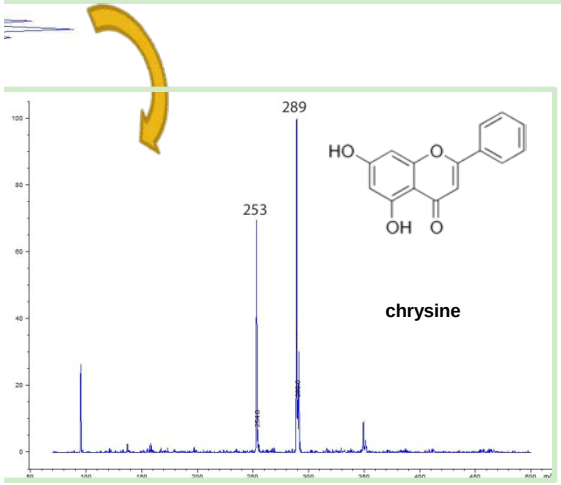
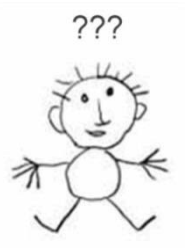
HPTLC-DART-MS:

Desorption-based HPTLC-MS

- ✓ DART-MS does not consume the complete analyte quantity from the plate
- ✓ possible to perform HPTLC/DART-MS and HPTLC/ESI-MS from the same plate, successively.



Conclusions



HPTLC-DART-MS

Scanning analysis across the plate section. No solvents are used, ionization takes place in the flow of gas

Semi-desctructive: possible to repeat the analysis from the same zone

Mass spectra: additional information due to the new mode of ionization

Quantitation capabilities are now higher due to the *x-y-z*-table

Sensitivity lacking

HPTLC-ESI-MS

Elution of selected zones by the solvent from HPLC pump

Destructive: cuts the zone of interest

Mass spectra contain sodium adducts and multiply charged ions

Quantitation is highly reliable, but accurate zone marking is essential

Sensitivity: generally 2-3 orders of magnitude better than for HPTLC-DART-MS

Our acknowledgements

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Dr. Dogan (Merck)

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Pete Ryan and Sue Kennerley (KR Analytical, UK)

Brian Musselman (IonSense, USA)

DAAD Deutscher Akademischer Austausch Dienst
German Academic Exchange Service

МИНИСТЕРСТВО ОБРАЗОВАНИЯ И НАУКИ
РОССИЙСКОЙ ФЕДЕРАЦИИ

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