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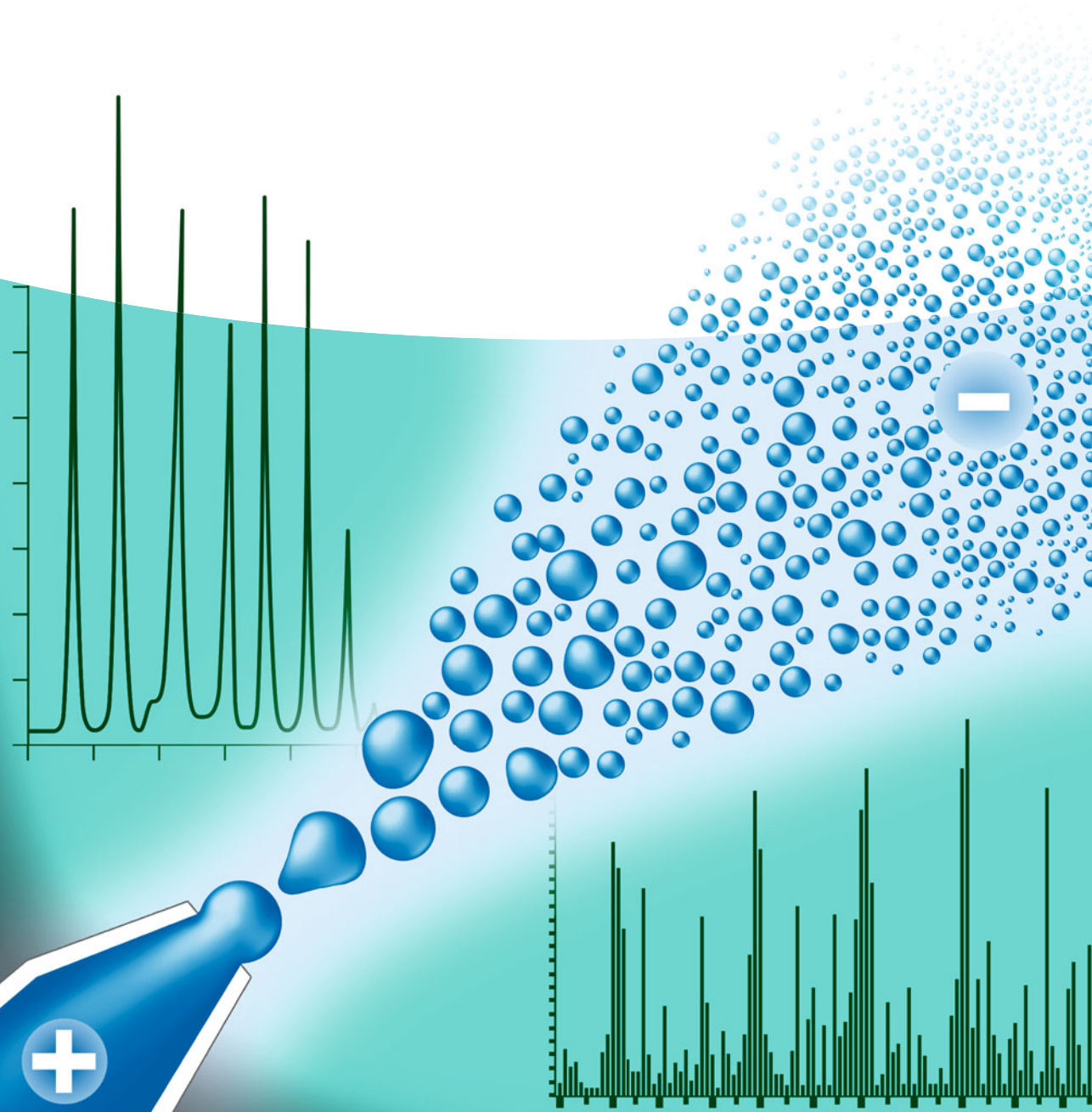
The HPLC-MS Handbook for Practitioners



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Preface

LC/MS coupling has developed from a method for experts in research to a well-proven technique for users in their daily routine. Hence, this book is dedicated exclusively to LC/MS coupling.

It is our goal to give LC/MS users detailed information in order to use *their* LS/MS application in an optimal manner. Colleagues who have authored articles in my previous books have therefore revised and updated their articles. Furthermore, new articles from LC/MS practitioners were added. When writing those articles, it was most important to us to have an eye on practice, but compact background knowledge is also given. I hope that the analyst in development as well as the user in daily routine will find inspiration and tips for optimal usage of LC/MS coupling.

My special thanks goes to Wolfgang Dreher for his critical comments to this manuscript, furthermore to my author colleagues, who put down their experience and knowledge in writing despite their limited time resources. I would like to thank WILEY-VCH and in particular Reinhold Weber and Martin Preuss for the good and close cooperation.

Blieskastel, March 2017 *Stavros Kromidas*

The Structure of *HPLC-MS for Practitioners*

The book contains ten chapters that are divided into four parts:

- **1–3: Part I Overview, Pitfalls, Hardware Requirements**
- **4: Part II Tips, Examples**
- **5–7: Part III User Reports**
- **8–10: Part IV Vendor's reports, Trends**

Part I

In Chapter 1 Oliver Schmitz overviews the **State of the art of LC/MS coupling** and opposes different modes. In Chapter 2 Markus Martin shows **Technical aspects and pitfalls of LC/MS hyphenation** and provides precise and specific hints how LC/MS coupling can successfully be established in daily routine. Other topics of Chapter 2 are method development as well as method transfer. Thorsten Teutenberg and co-authors provide a great many of suggestions in Chapter 3 (**Requirements of LC hardware for the coupling of different mass spectrometers**), as to arrange LC/MS coupling as optimal as possible. Among other things complex samples and miniaturization play an important role.

Part II

In Chapter 4 Friedrich Mandel offers a numerous of **LC/MS Tips** addressing different topics of LC/MS coupling.

Part III

Chapter 5 contains examples and experience reports from users and service engineers: LC/MS coupling is often linked to life science and environmental analysis. Alban Muller and Andreas Hofmann show in Chapter 5 a **concrete example of LC/MS coupling in ion chromatography** as an unfamiliar application. In Chap-

ter 6 Edmond Fleischer shows on the basis of 4 examples coming from the field of synthesis how to proceed if characterization of impurities are on focus (**Problem solving with HPLC-MS – a practical view from practitioners**). Oliver Müller (**LC/MS from the perspective of a maintenance engineer)** undertakes a virtual walk across a MS and gives hints how to handle the problem "impurities in LC/MS".

Part IV

Finally, in Part IV (Chapter 8–10, **Report of device manufacturers – article by Agilent, SCIEX, and ThermoScientific**) three manufacturers introduce briefly their newest products and evaluate the future of HPLC-MS coupling.

We think the style and structure of *The HPLC Expert* has proven itself, so those were kept the same in the subsequent book: the book need not to be read linearly. All chapters present self-contained modules – "jumping" between chapters is always possible. That way we try to keep the character of the book as a reference book for LC/MS users. The reader may benefit therefrom.

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Part I
Overview, Pitfalls, Hardware-Requirements

1
State of the Art in the LC/MS

O. Schmitz

1.1
Introduction

The dramatically increased demands on the qualitative and quantitative analysis of more complex samples are a huge challenge for modern instrumental analysis. For complex organic samples (e.g., body fluids, natural products or environmental samples), only chromatographic or electrophoretic separations followed by mass spectrometric detection meet these requirements. However, at the moment a tendency can be observed, in which a complex sample preparation and preseparation is replaced by high-resolution mass spectrometer with atmospheric pressure ion sources. However, numerous ion–molecule reactions in the ion source – especially in complex samples due to incomplete separation – are possible because the ionization in typical atmospheric pressure ion sources is nonspecific [1]. Thus, this approach often leads to ion suppression and artifact formation in the ion source, particularly in electrospray ionization (ESI) [2].

Nevertheless, sources such as ASAP (atmospheric pressure solids analysis probe), DART (direct analysis in real time), and DESI (desorption electrospray ionization) can often be successfully used. In ASAP, a hot nitrogen flow from an ESI or APCI (atmospheric pressure chemical ionization) source is used as a source of energy for evaporation and the only change to an APCI source is the installation of an insertion option to place the sample in the hot gas stream within the ion source [3]. This ion source allows a rapid analysis of volatile and semivolatile compounds and, for example, was used to analyze biological tissue [3], polymer additives [3], fungi and cells [4], and steroids, [3, 5]. ASAP has much in common with DART [6] and DESI [7]. The DART ion source produces a gas stream containing long-lived electronically excited atoms that can interact with the sample and, thus, desorption and subsequent ionization of the sample by Penning ionization [8] or proton transfer from protonated water clusters [6] is realized. The DART source is used for the direct analysis of solid and liquid samples. A great advantage of this source is the possibility to analyze compounds on surfaces such as illegal substances on dollar bills or fungicides on wheat [9]. Unlike ASAP and DART, the great advantage of DESI is that the volatility of the analyte is not a

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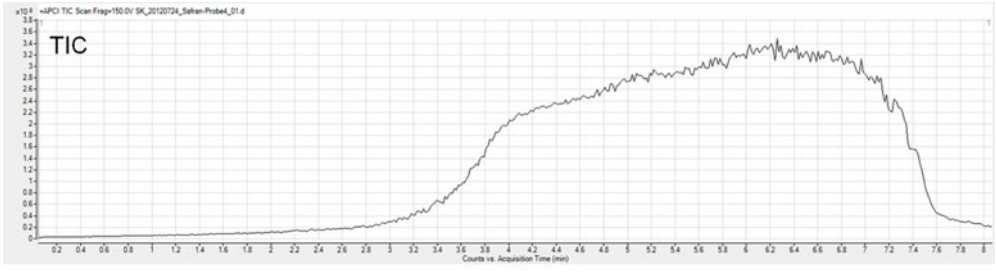
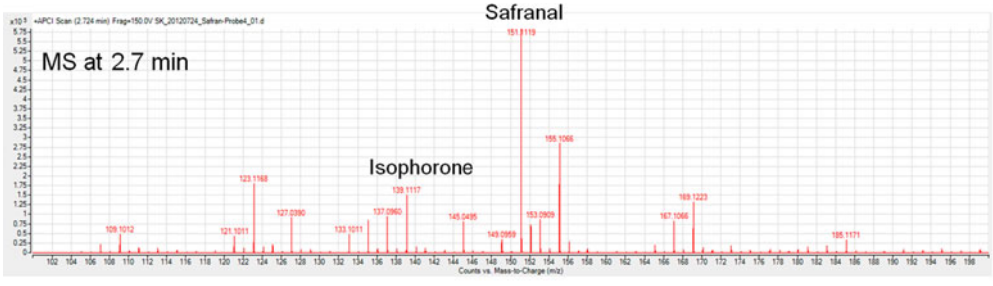


(a)



(b)

Figure 1.1 Analysis of saffron using direct-inlet probe-APCI with high-resolution QTOF-MS. (a) TIC of the toal analysis. (b) mass spectrum at the time of 2.7 min.

prerequisite for a successful analysis (same as in the classic ESI). DESI is most sensitive for polar and basic compounds and less sensitive for analytes with a low polarity [10]. These useful ion sources have a common drawback. All or almost all substances in the sample are present at the same time in the gas phase during the ionization in the ion source. The analysis of complex samples can therefore lead to ion suppression and artifact formation in the atmospheric pressure ion source due to ion–molecule reactions on the way to the MS inlet. For this reason, some ASAP applications are described in the literature with increasing temperature of the nitrogen gas [5, 11, 12]. DART analyzes with different helium temperatures [13] or with a helium temperature gradient [14] have been described in order to achieve a partial separation of the sample due to the different vapor pressures of the analyte. Related with DART and ASAP, the direct inlet sample APCI (DIP-APCI) from Scientific Instruments Manufacturer GmbH (SIM) was described 2012, which uses a temperature-push rod for direct intake of solid and liquid samples with subsequent chemical ionization at atmospheric pressure [15]. Figure 1.1 shows a DIP-APCI analysis of a saffron sample (solid, spice) without sample preparation with the saffron-specific biomarkers isophorone and safranal. As a detector, an Agilent Technologies 6538 UHD Accurate-Mass Q-TOF was used. The total ion chromatogramm (TIC) of the total analysis and the mass spectrum at the time of 2.7 min are shown in Figure 1.1a,b, respectively. The analysis was started at 40 °C and heated the sample at 1 K/s to a final temperature of 400 °C.

These ion sources may be useful and time saving but for the quantitative and qualitative analysis of complex samples a chromatographic or electrophoretic preseparation makes sense. In addition to the reduction of matrix effects, the comparison of the retention times also allows an analysis of isomers.

1.2 Ionization Methods at Atmospheric Pressure

In the last 10 years, several new ionization methods for atmospheric pressure (AP) mass spectrometers have been developed. Some of these are only available in some working groups. Therefore, only four commercially available ion sources will be presented in detail here.

The most common atmospheric pressure ionization (API) is electrospray ionisation (ESI), followed by APCI and APPI (atmospheric pressure photoionization). A significantly lower significance shows the APLI (atmospheric pressure laser ionization). However, this ion source is well suited for the analysis of aromatic compounds and, for example, the gold standard for PAH (polyaromatic hydrocarbons) analysis. This ranking reflects more or less the chemical properties of the analytes, which are determined with API MS: Most analytes from the pharmaceutical and life sciences are polar or even ionic and, thus, are efficiently ionized by ESI (Figure 1.2). However, there is also a considerable interest in API techniques for efficient ionization of less or nonpolar compounds. For the ionization of such substances ESI is less suitable.

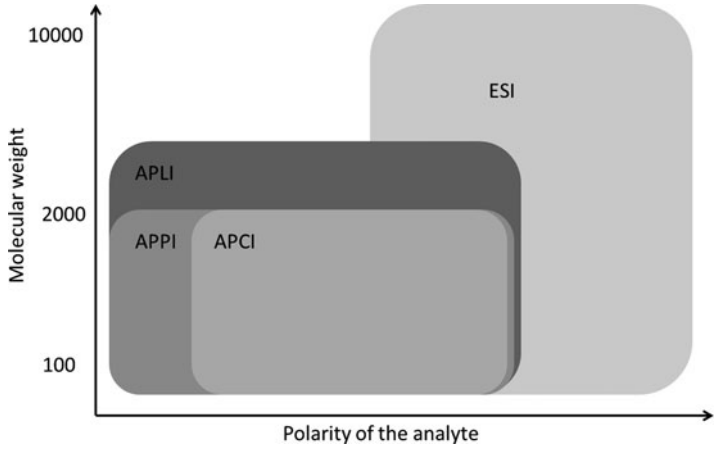


Figure 1.2 Polarity range of analytes for ionization with various atmospheric pressure ionization (API) techniques. Note: The extended mass range of APLI against APPI and APCI results from the ionization of nonpolar aromatic analytes in an electrospray Reproduced with kind permission of O. J. Schmitz, T. Benter, Advances in LC-MS Instrumentation: Atmospheric pressure laser ionization, Journal of Chomatography Libary, Vol 72 (2007), Chapter 6, Pages 89-113.

1.2.1 Overview of API Methods

Ionization methods that operate at atmospheric pressure, such as atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI), have greatly expanded the scope of mass spectrometry [17–20]. These API techniques allow an easy coupling of chromatographic separation systems, such as liquid chromatography (LC), to a mass spectrometer.

There is a fundamental difference between APCI and ESI ionization mechanism. In APCI, ionization of the analyte takes place in the gas phase after evaporation of the solvent. In ESI, the ionization takes place already in the liquid phase. In the ESI process, protonated or deprotonated molecular ions are usually formed from highly polar analytes. Fragmentation is rarely observed. However, for the ionization of less polar substances, APCI is preferably used. APCI is based on the reaction of analytes with primary ions, which are generated by corona discharge. But the ionization of nonpolar analytes is very low with both techniques.

For these classes of substances other methods have been developed, such as the coupling of ESI with an electrochemical cell [21–32], the "coordination ion-spray" [32–47] or the "dissociative electron-capture ionization" [38–42]. The atmospheric pressure photoionization (APPI) or the dopant-assisted (DA) APPI presented by Syage *et al.* [43, 44] and Robb *et al.* [45, 46], respectively, are relatively new methods for photoionization (PI) of nonpolar substances by means of vacuum ultraviolet (VUV) radiation. Both techniques are based on photoionization, which is also used in ion mobility mass spectrometry [47–50] and in the photo ionization detector (PID) [51–53].

1.2.2 ESI

In the past, one of the main problems of mass spectrometric analysis of proteins or other macromolecules was that their mass was outside the mass range of most mass spectrometers. For the analysis of larger molecules, such as proteins a hydrolysis and the analysis of the resulting peptide mixture had to be carried out. With ESI it is now possible to ionize large biomolecules without prior hydrolysis and analyze them by MS.

Based on previous works from Zeleny [54], Wilson and Taylor [55, 56], Dole *et al.* produced high molecular weight polystyrene ions in the gas phase from a benzene/acetone mixture of the polymer by electrospray [57]. This ionization method was finally established through the work of Fenn in 1984 [58], who was awarded the Nobel Prize for Chemistry in 2002.

In order to describe the whole process of ion formation in ESI, a subdivision of processes into three sections makes sense:

- Formation of charged droplets
- Reduction of the droplet
- Formation of gaseous ions.

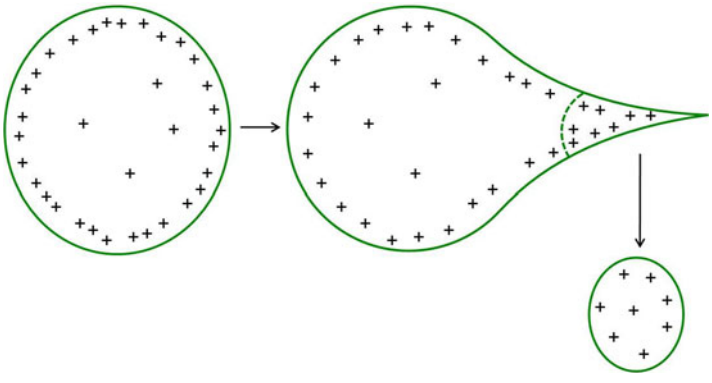


Figure 1.3 Reduction of the droplet size.

To generate positive ions a voltage of 2–3 kV between the narrow capillary tip (10^{-4} m outer diameter) and the MS input (counter electrode) is applied. In the exiting eluate from the capillary, charge separation occurs. Cations are enriched at the surface of the liquid and moved to the counter electrode. Anions migrate to the positively charged capillary, where they are discharged or oxidized. The accumulation of positive charge on the liquid surface is the cause of the formation of a liquid coned, as the cations are drawn to the negative pole, the cathode. This so-called Taylor cone resulted from the electric field and the surface tension of the solution. With certain distance from the capillary, there is a growing destabilization and a stable spray of drops with an excess of positive charges will emitted.

The size of the droplets formed is dependent on the

- Flow rate of the mobile phase and the auxiliary gas
- Surface tension
- Viscosity
- Applied voltage and
- Concentration of the electrolyte.

These drops lose solvent molecules by evaporation and at the Raleigh limit (electrostatic repulsion of the surface charges > surface tension) much smaller droplets (so-called microdroplets) are emitted (Figure 1.3). This occurs due to elastic surface vibrations of the drops which lead to formation of Taylor cone-like structures.

At the end of such protuberances small droplets are formed, which have a significantly smaller mass/charge ratio than the "mother drop". Because of the unequal decomposition the ratio of surface charge to the number of paired ions in the droplet increases dramatically per cycle of droplet formation and evaporation up to the Raleigh limit in comparison with the "mother drops". Thus, only highly charged microdroplets are responsible for the successful formation of ions.

For the ESI process, the formation of multiply charged ions for large analyte molecules is characteristic. Therefore, a series of ion signals for, for example, pep-