Trace analysis by HPLC-MS: contamination problems and systematic errors

Michael Oehme*, Urs Berger, Stephan Brombacher, Fabian Kuhn, Stephen Kölliker
Organic Analytical Chemistry, University of Basel, Neuhausstr. 31, CH-4057 Basel, Switzerland

HPLC-MS has been introduced into many laboratories during the past five years. However, this methodology is much more demanding than HPLC with optical detection or GC-MS. HPLC separations are still not optimised for MS and trace analysis. When working with trace quantities, there is considerable risk of problems caused by adsorption, contamination, surface reactions or other interferences. Transfer lines and ionisation processes can also cause systematic errors. This review summarises possible sources of error in HPLC-MS and suggests how to avoid them. Examples are given to illustrate problems. © 2002 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

During the past decade, high-performance liquid chromatography (HPLC) coupled to mass spectrometry (MS) – HPLC-MS – has grown up. The main reasons are robust ionisation techniques and the possibility of taking advantage of the whole eluent flow from a HPLC column of 2–4 mm internal diameter [1].

Currently, atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI) are used most frequently [2]. Furthermore, detection limits are comparable to those of the well-established combination of gas chromatography (GC) with MS - GC-MS. The selected ion monitoring (SIM) mode allows the detection of quantities in the picogram (pg) range with quadrupoles, and an ion trap mass spectrometer can record full mass spectra with 1 nanogram (ng) or less. The latter was introduced in 1996 [3]. It is also able to gain structure information by trapping selected fragments, followed by fragmentation and registration of the resultant ion spectra. This can be repeated a number of times in succession, and this is known as multiple MS (abbreviated as MSn [3,4]).

Because of the improved detection limits and possibility of structure elucidation ([5,6] give examples), the number of users of HPLC-MS has increased appreciably in recent years. Many of them had previous experience in HPLC with various detection methods or GC-MS. However, the know-how gained with these techniques cannot be transferred directly to HPLC-MS. The main reasons are: the almost universal detectability of compounds using MS, when compared to UV detection; the different properties of the applied ionisation techniques; and, the incompatibility between MS and eluents containing certain types of buffers (such as phosphate buffers).

In addition, stationary phases for reversed phase HPLC often show some hydrolysis of the bound lipophilic rests, which leads to increased background. Furthermore, the phase surface still has a relatively high number of active sites, which pose a risk of compound adsorption and degradation at trace levels (in the pg to ng
range). People with GC-MS experience will soon appreciate that LC-MS is a far less robust technology, despite the claims of some instrument manufacturers.

The factors mentioned above increase the possibility of systematic errors and artefacts during quantification and identification at trace levels. Nearly any element and any operation of HPLC can introduce contaminants or cause interferences. Interestingly, as also experienced in GC-MS, the mass spectrometer can be blamed as source of problems in the minority of cases only. Some examples of effects leading to false positive or negative results are as follows:

- Suppression of compound ionisation by a more easily ionisable background or by coelution of another substance (not visible in the SIM mode). The background can be caused by the sample matrix, a buffer or column bleeding. ESI is especially sensitive to such effects.
- Contaminants in solvents and water used for gradients can also give a high background. Most HPLC-grade quality solvents and waters are tested for UV transparency only and not with respect to a low MS background.
- Ion-molecule adducts can be formed between analytes and alkaline metal ions or ammonium. Their yields vary with the salt content in the sample and salt residues built up in the spray region. Here too ESI is particularly sensitive to such adduct formation.
- Misalignments in instruments can cause problems, such non-linearity through the wrong positioning of the corona discharge needle in APCI.
- Even degassing of solvents or a transfer line made from quartz may result in unexpected disturbances.

Though many users face such difficulties in their daily work, information about artefacts is scarce in the literature. Perhaps, it is not sufficiently scientific to describe irritating and time-consuming problems, so the intention of this article is to summarise some problems observed in our research group in the field of trace analysis (pg to ng range) by HPLC-MS and their consequences for compound quantification and detection.

2. Experimental conditions

The examples described in the following sections were recorded under different separation and ionisation conditions. However, common parameters may be briefly summarised as follows:

- A low pressure binary gradient HPLC pump (Rheos 4000, Flux Instruments, Basel, Switzerland) was employed.
- The samples were injected with a Valco Cheminert valve equipped with a 5 μL to 25 μL loop (stainless steel or PEEK).
- Reversed phase HPLC separation was applied (for example on a C18 normal density phase [Nucleosil, 100 Å pore size, 3 μm particles, 125 mm column length, 3 mm i.d., Macherey-Nagel, Oensingen, Switzerland]).
- The flow rate of the mobile phase was around 250–600 μL/min. Binary gradients of water/methanol or water/acetonitrile were employed.
- An ion trap mass spectrometer (LCQ Classic, Finnigan MAT, San Jose, CA, USA) was used in the positive (+) or negative (−) ion mode employing APCI or ESI.
- Mass spectra were registered in the full scan mode (mass range 100–200 u to 500–2000 u).
- The following APCI parameters were applied: heater temperature, 230–450 °C; nitrogen sheath gas flow, 25–40 arbitrary units (corresponding to about 250–400 mL/min); ionization current of corona discharge, 1.5–5 μA.

3. Problems caused by contamination

In general, the chromatographic background in HPLC-MS is higher than in GC-MS because
of higher consumption of the mobile phase and the transfer of impurities therein. As a result, HPLC-MS chromatograms are usually presented in the base ion (BI) mode and not as total ion chromatograms (TIC).

Under BI conditions, only the abundance of the BI in the mass spectrum is selected to generate a chromatogram. As can be seen (Fig. 1), this allows the visualisation of compounds at quantities of about 100 pg that otherwise are completely overlaid by the background in the TIC. Fig. 1 also demonstrates the detection performance of ion trap mass spectrometers in the full scan mode.

Compared to HPLC-UV detection, where water for gradients should be free of any UV-absorbing material only, a much better, more comprehensive pre-cleaning is needed for HPLC-MS. Only water with a total organic carbon content of ≤2ppb is good enough, and it can be obtained by combining osmosis, ion exchange, activated charcoal adsorption, degradation by UV, and adsorption on charcoal again. Such systems are commercially available but they need conditioning for several days before they reach the good quality required. Fig. 2 shows the APCI BI chromatogram in the negative ion mode of the water background after the cleaning cartridges have been replaced. None of the contaminants could be detected by HPLC-UV. After two days of recirculating water, the contamination had disappeared.

In general, the negative ion mode is much more sensitive to contamination problems, as

![Fig. 1. Comparison of an APCI(-) total ion (A) and base ion chromatogram (B) of 2,4-dinitrophenyl hydrazones of carbonyl compounds present in ambient air. Shown concentrations in (B) range from ca. 100 pg (e.g. no. 7, 150 pg) to 10–20 ng (no. 20). Major compounds are (2), (6), (17), (20) and (21). For more information, see [7].](image-url)
also demonstrated by the APCI(-) B1 chromatogram of an UV-grade acetonitrile with polar impurities, which formed a high background by polymerisation after ionisation. The polar impurities coated even the APCI corona discharge needle with soot so that the discharge current collapsed (Fig. 3A). A simple distillation solved the problem.

Ghost signals can also be generated by solvent degassing. Units based on semi-permeable tubing (often more than 100 m long) can release contaminants as a result of microbial growth. Degassing by He can also introduce impurities from pressure reduction valves and tubing, as demonstrated in Fig. 4. As a result, gas purification by a charcoal filter is highly recommended.

Even HPLC pumps are sometimes not clean enough. Fig. 5 shows a APCI(+) background mass spectrum with high levels of silicones and dioctylphthalate. This indicates contamination by silicone tubing or membranes – in this case, a silicon tube at the piston back flush inlet. The impurities migrated slowly via the piston seals into the eluent.

As demonstrated [8], a rather variable resistance of stationary phases against hydrolysis can also contribute to a substantial background, particularly in the APCI(-) mode. When not rinsed by a slow flow of mobile phase (for example overnight or during storage), alkylsiloxane rests are cleaved off from alkyl chain-modified stationary phases by continuous hydrolysis. These residues accumulate in the column. Depending on their quantity, it can take minutes or hours for flushing to remove them. Huge differences were observed between commercial columns. Those tightly packed and showing a high separation efficiency had a tendency to exhibit higher column bleed, probably as a result of fragmented particles forming new active surfaces.

4. Problems caused by adsorption

Irreversible adsorption on stationary phases is particularly feared as a source of systematic errors. Chromatographic signals are then smaller, but still of good peak form, as opposed to those obtained with reversible adsorption, where signal tailing is observed. HPLC-MS detection limits in the pg range have increased this risk, since the large, still relatively active surface of HPLC column packings might show

![Fig. 2. APCI(-) base ion chromatogram of water contamination after exchange of cartridges in a complex water-purification system (see text). Recirculation of water for two days was necessary before the HPLC-MS background became satisfactory again.](image-url)
adsorption effects at such low concentrations. Fig. 6 demonstrates this.

Surfaces of new columns may contain many active sites, which have to be saturated by “priming” (with several injections of high levels of compounds). Signal areas may also increase over time, as comparison of the signal sizes of injection no. 1 and 200 show for the rather polar biotoxins nivalenol and deoxynivalenol, which are often present in cereals [6].

Adsorption of polar compounds might also occur on active surfaces of transfer lines. In an example [8], aconitum alkaloids are strongly adsorbed on a fused silica tube between column outlet and ESI ion source. This problem was solved by adding to the eluent millimolar amounts of ammonium acetate, which saturated

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**Fig. 3.** Non-separable impurities in HPLC-grade acetonitrile resulted in a polymerisation reaction leading to soot deposition and collapse of the corona discharge current shortly after 36 min. A: APCI(-) base ion chromatogram, column Nucleosil C18 HD, 3 μm particles, 100 Å pore size, 125 mm length × 3 mm ID, CH₃CN/H₂O 50+50, in 37.5 min to 100% CH₃CN, 8 min isocratic, flow 560 μL/min. B: Averaged APCI(-) background mass spectrum at 34–35.5 min. C: APCI(-) base ion chromatogram of same scale after distillation.
Fig. 4. Contaminants introduced by solvent degassing with He. A: APCI(+) base ion chromatogram, column Nucleosil C_{18} HD, 3 μm particles, 100 Å pore size, 125 mm length × 3 mm ID, CH₃OH/H₂O 25+75, in 12 min to 100% CH₃OH, flow 560 μL/min. B: APCI(+) mass spectrum of impurity at 10.4 min. C: APCI(+) base ion chromatogram without degassing.
active sites, so that the peak shapes were no longer distorted.

5. Problems caused by compound ionisation

Fig. 7 presents non-linear calibration curves for the 2,4-dinitrophenylhydrazone derivatives of glyoxal (ethanediol) and methyl-glyoxal (oxopropanal) in the APCI(-) mode. This problem started after cleaning the APCI source, and the non-linearity was highly reproducible. Detailed inspection of the source revealed that the corona discharge needle was about 4–7 mm out of centre relative to the heater. No precise adjustment aids or information about such effects had been given by the instrument supplier. The very slight off-axis formation of the plasma was serious enough to cause a general decrease in sensitivity and a concentration-dependent transfer of the formed ions. The problem was solved by correcting the needle position.

Ionisation yield in LC-MS may also vary more than in GC-MS. Run-to-run deviations of 10% are frequently observed and might introduce a substantial measurement uncertainty. As a result, the method of choice should be quantification relative to an internal standard, which is added to the sample just before quantification. If internal standards are selected with similar compound properties, linearity with absolute deviations of <10% is possible over three orders of magnitude.

So far, our experience is that, for quantification, APCI is superior to ESI, since the sample matrix influences the ionisation yield much less. Stable ion-molecule adducts with salt ions and ionisation suppression by large biomolecules are the main reasons for the risk of systematic errors in ESI being higher. Fig. 8 shows an example of extensive ion-molecule adduct formation for a larger biomolecule. This makes quantification very demanding, if not impossible.

Another problems, detected just recently, is adduct formation between solvent molecules and daughter ions obtained by collision-induced dissociation in an ion trap. Solvent-specific adducts were found in the MS3 spectrum of propanone DNPH for the sequence [M-H]⁻ → m/z 151 → scan. Adducts with methanol (m/z 183), acetonitrile (m/z 192) or ethanol (m/z 197) could be detected and the completely changed the appearance of the mass spectra [9]. This has consequences for both the establishment of

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**Fig. 5.** APCI(+) background mass spectrum with contaminants from a silicone tube mounted at the piston backflush unit. Contaminants, such as dioctylphthalate and silicones, migrated via piston seals into the eluent.
Fig. 6. APCI(-) base ion chromatograms of the separation of ca. 5 ng of 2,4-dinitrophenyl-hydrazone derivatives of C₁-C₈ carbonyls. A: New column. B: Same column after 10 injections. APCI(+) base ion chromatograms of the separation of ca. 2.5–3.5 ng of trichothecenes. C: New column. D: Same column after 200 injections. Column Nucleosil C₁₈ ND, 5 µm particles, 100 Å pore size, 125 mm length, 3 mm ID. NIV: Nivalenol, DON: Deoxynivalenol, for further abbreviations, see [6].
Fig. 7. APCI(-) calibration curves for glyoxal and methylglyoxal with a slightly misaligned corona discharge needle (see small figure, 4–7 mm off) and one correctly positioned (large figure).

Fig. 8. Ion molecule adduct formation of Leucine Enkephalin with K⁺ and Na⁺ in the ESI mode. The formation of a large number of adducts with a yield varying with the background salt concentration makes compound identification and quantification difficult.
libraries of daughter ion spectra and the interpretation of mass spectra.

6. Possibilities for detecting systematic errors in LC-MS

All the problems described above may introduce systematic errors that are related to either matrix effects in single samples or failure to optimise the method. Normal quality-control measures are not well suited to detecting systematic errors. Neither good precision nor high recovery of an extraction standard guarantees that there are no systematic deviations. Inter-calibrations, where a great variety of methods is used, might give information about systematic deviations. However, since the true value is normally not known, it is not possible to draw any conclusions. Unfortunately, some “round robin” tests require use of the same method. Such comparisons do nothing more than control the robustness of the method and are definitively not suited to detecting systematic errors.

Control of the quality of newly developed LC-MS methods with certified materials should also be interpreted with caution. In the past, HPLC with UV or fluorescence detection was often employed for certification, as in some cases was GC-MS after derivatisation. Because of the restricted number of really independent techniques, systematic deviations might be detected when examining the test material by LC-MS for the first time. In the past, incorrect levels have occurred for reference materials certified by less selective methods [10]. As a result, such systematic deviations should be considered a real possibility as long as no reasonable explanation can be found.

The possibility of detecting systematic errors in one’s own laboratory are limited, but often even those tests that are possible are not applied. Sample-matrix effects quite often cause artefacts. One powerful possibility is a multiple standard addition of known quantities of analytes to samples. Since the influence of the sample matrix usually decreases with increasing analyte quantity, a linear relation between concentration and signal area might be observed at higher levels and deviations at lower ones. Spiking a calibration solution with an increasing background of sample matrix is a further possibility for detecting any influence of the sample matrix on the ionisation yield. The response factor should remain unchanged, if no disturbances are present.

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