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Use of evaporative light scattering detection for the quality control of drug substances: Influence of different liquid chromatographic and evaporative light scattering detector parameters on the appearance of spike peaks

Stefan Almeling[a], Ulrike Holzgrabe[b,*]

[a] European Directorate for the Quality of Medicines and Health Care, Allée Kastner, 67081 Strasbourg, France
[b] Institute of Pharmacy and Food Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

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ABSTRACT

High performance liquid chromatography (HPLC) with evaporative light scattering detection (ELSD) is a versatile, easy to use and inexpensive alternative when it comes to the analysis of substances lacking a chromophor for UV detection. However, in pharmaceutical analysis injection of highly concentrated test solutions are normally required to control impurities at low levels. Under these conditions spike peaks were observed in the chromatograms of the test solutions making a proper evaluation of the impurity profile impossible. The influence of different eluent and ELSD parameters such as eluent composition, eluent flow-rate, ELSD scavenger gas flow-rate and evaporation temperature on the appearance of spike peaks was investigated. It could be shown that spike peaks can be avoided when selecting elevated eluent flow-rates and ESLD scavenger gas flow-rates. Moreover, eluents containing high amounts of organic modifier seem to foster the appearance of spike peaks.

1. Introduction

A key objective in the quality control of active pharmaceutical ingredients (API) and excipients used for the production of drug products is an appropriate control of impurities often referred to as related substances. In guideline Q3A(R) on the control of impurities in new drug substances [1], the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has set a reporting threshold of 0.05% for impurities in new drug substances with an average daily dose below 2 g and 0.03% above this limit. Impurities exceeding this threshold must be quantified and reported. In the European Pharmacopoeia (Ph.Eur.) [2] the above concept is not limited to new drug substances, but was extended to all substances for pharmaceutical use. However, the Ph.Eur. refers to the *reporting threshold* as *disregard limit*. As a consequence, methods used for the control of impurities must exhibit a limit of quantification which corresponds at least to the *reporting threshold* or *disregard limit*. Hence, the method employed for the control of impurities in "substances for pharmaceutical use" need to be sufficiently sensitive to comply with the above requirement.

The technique most used for control of impurities in the Ph.Eur. is liquid chromatography (LC) with UV detection. The main advantage of UV detection is that it is easy to use. Nonetheless, UV detection comes to its limits when dealing with molecules that lack a chromophor. In these cases refractive index (RI), electrochemical (EC) detection or post-column derivatisation UV detection is often employed instead. However, all alternatives have limitations [3]: refractometry is not sufficiently sensitive and is not suitable for gradient elution. Amperometric detection requires frequent cleaning of the electrodes due to poisoning. Derivatisation is difficult to validate as the impurities may interact differently with the derivatisation agent. In contrast, evaporative light scattering detection (ELSD) could represent a convenient alternative detection mode [3–6]. This is particularly true when the amounts of the analytes to be determined are not too different from one another, as is the case e.g. for the drug gentamicin which is composed of five closely related components of 2–40% in addition to the impurities each of less than 3% [7–10].

A comparative study of different LC detectors performed by Petritis et al. [11] for the analysis of underivatized amino acids, revealed several advantages of the ELSD, e.g. gradient compatibility. However, one restriction when using ELSD, as it is also the case for mass-detection, is the need of a volatile eluent. Moreover, it must be kept in mind that the ELSD response is not linear.

An attempt was made by Kopec [12] to develop a method for the control of impurities in L-alanine and Asp by a LC ion-pair

* Corresponding author. Tel.: +49 931 888 5460; fax: +49 931 888 5494.
E-mail address: u.holzgrabe@pharmazie.uni-wuerzburg.de (U. Holzgrabe).

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Table 1
Overview of the applied experimental conditions.

LC mobile-phase composition	LC flow-rate	ELSD scavenger gas flow-rate	ELSD drift tube temp.	ELSD nebulizer temp.
(2.3.1) Variation of the mobile-phase composition				
Test solutions: (1) and (2)				
(a) Water/acetonitrile [(90/10, v/v) to (20/80, v/v) modified in 10% steps]	0.5 mL/min	1.0 standard liter per minute (SLM)	50 °C	50 °C
(b) Water/methanol [(90/10, v/v) to (20/80, v/v) modified in 10% steps]	0.5 mL/min	1.0 SLM	50 °C	50 °C
(2.3.2) Variation of the eluent flow-rate				
Test solutions: (1) and (3); test solution 2 only under conditions (a)				
(a) Water/methanol (80/20, v/v)	0.5–0.7–0.8–1.0–1.2–1.5 mL/min	1.0 SLM	50 °C	50 °C
(b) Water/acetonitrile (90/10, v/v)	As above + 2.0 mL/min	1.0 SLM	50 °C	50 °C
(2.3.3) Variation of the ELSD scavenger gas flow-rate				
Test solutions: (1) and (3)				
(a) Water/methanol (80/20, v/v)	0.5–0.7–0.8–1.0–1.2–1.5 mL/min	0.9–1.0–1.1–1.2–1.4 SLM	50 °C	50 °C
Test solution (2)				
(b) Water/methanol (50/50, v/v)	As under 2.3.3(a)	0.9–1.0–1.1–1.2–1.4–1.8–2.2 SLM	50 °C	50 °C
(c) Water/methanol (20/80, v/v)	As under 2.3.3(a)	As under 2.3.3(b)	50 °C	50 °C
(2.3.4) Variation of the nebulizer and evaporation (drift tube) temperature				
Test solution: (1)				
(a) Water/methanol (80/20, v/v)	0.5 mL/min	1.0 SLM	40–50–60–70 °C	
(b) Water/methanol (80/20, v/v)	0.5 mL/min	1.0 SLM	40–50–60–70–80 °C	30 °C

chromatographic method using ELSD detection. However, when injecting the concentrated test solutions non-reproducible spikes[1] appeared after the main peak, making an appropriate identification and quantification of potential impurities impossible. The same phenomenon was reported when other substances, e.g. caffeine, fumaric acid, or spectinomycin were injected. An influence of the LC column could be excluded, since the spikes also appeared when injecting the test solutions without a column. Interestingly, the appearance of spike peaks[1] hampering detection of analytes in LC with ELSD is hardly reported in literature.

For a possible use of the ELSD for the impurities control in "substances for pharmaceutical use", a pre-requisite is that the analytical system tolerates high concentrations of the active substance and is still capable of detecting impurities at very low levels. Hence, the purpose of this study was to investigate the influence of different ELSD and eluent parameters on the appearance of peak spikes. Moreover, possibilities to avoid the occurrence of spike peaks were explored.

2. Experimental

2.1. Reagents and chemical

Water was delivered by an ELGA PureLab Ultra system Elga Antony, France. Diluted hydrochloric acid (0.37 g/L) was prepared starting from hydrochloric acid 37% p.a. (Merck, Darmstadt, Germany). Methanol puriss. p.a., acetonitrile puriss. p.a., and L-aspartic acid (Asp) +99% were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany), mexiletine hydrochloride from ICN (Budapest, Hungary), and α-cyclodextrin (alfadex) was provided by Roquette (Lestrem, France). Nitrogen +99% was delivered by a Peak Systems NM18LA nitrogen generator (Lab Gaz Systems, Massy, France).

2.2. Apparatus

A Waters Alliance Separation Module 2695 consisting of autosampler, injector and quarternary pump (St. Quentin-en-Yvelines, France) equipped with Waters Empower Pro data processing software was used for flow injection analysis. Detection was performed by a Polymer Laboratories PL-ELS 2100 Evaporative Light Scattering Detector (Marseille, France) using nitrogen as scavenger gas. All analyses were run with a peek-tube 1/32 inch restriction coil having an internal diameter of 125 μm and a length of 19 cm (Interchim, Montlucon, France).

2.3. Flow injection analysis (FIA)

In 4 sets of experiments, the influence of the subsequent eluent composition and flow, and ELSD parameters on the appearance of spike peaks was examined:

(2.3.1) Variation of the eluent composition
(2.3.2) Variation of the eluent flow-rate
(2.3.3) Variation of the ELSD scavenger gas flow-rate
(2.3.4) Variation of the nebulizer and evaporation (drift tube) temperature

The experiments were carried out using as test solutions (1) 40 mg/mL Asp (prepared by dissolution of about 400 mg Asp in 2 mL of dilute hydrochloric acid (0.37 g/L) and dilution to 10.0 mL with

[1] The terms "spikes" and "spike peaks" in the context of this paper was chosen to describe the phenomenon of non-reproducible peaks eluting after the principal peak in concentrated analyte solutions.

water), (2) 40 mg/mL mexiletine HCl in water/methanol (50/50, v/v), and (3) 40 mg/mL alfadex in water. For a sensitivity check, 1.0 mL of the test solutions was diluted to 100.0 mL with water. 1.0 mL of this solution was further diluted to 20.0 mL with water (conc. 0.05%). In all experiments an injection volume of 40 μL and an ELSD detector gain of 1.0 was employed. To ensure that all possible spike peaks elute within the runtime and do not influence the subsequent injection, a run-time of 10 min was selected for the concentrated solutions. For the diluted solutions (0.05%) a run-time of 1.5 min was considered sufficient.

Table 1 gives an overview of the different eluent composition, eluent flow and ELSD parameters used in the experiments.

3. Results and discussion

The ELSD is considered to be a universal detector in liquid chromatography, provided the solute is considerably less volatile than the solvent at the operating temperature [13]. It measures the amount of light scattered by particles of the eluent that have been dried through the evaporation. In general, ELSDs deliver a signal for all compounds that do not evaporate during the mobile-phase evaporation stage [14]. The principle of detection can be summarized in the following steps: nebulization of the eluate, evaporation of the solvent and light scattering by the residual volatile particles including the analyte. Each process contributes to the overall response of the detector [3].

Since the aim of this study was to investigate the dependence of the appearance of spike peaks on different eluent composition, eluent flow and ELSD, settings, all tests were performed as FIA, using standard LC equipment without analytical column. To obtain sufficient system pressure, a restriction coil was used instead. The concentration of the test solutions was chosen in a way that a solution at a concentration of 0.05% of the concentration of the test solution would still deliver a signal above the limit of quantification (= signal-to-noise ratio of 10). In the analysis of drug substances this limit is normally referred to as “disregard limit” since impurities below this limit usually can be disregarded whilst those above must be reported. Since it was found within the first series of experiments, that this is not always guaranteed using a 20 mg/mL test solution, the concentration was increased to 40 mg/mL.

3.1. Influence of eluent composition, eluent flow and ELSD settings on the detector response

The response of the ELSD depends on several parameters, i.e. eluent composition, eluent flow-rate, ELSD scavenger gas flow-rate and the nebulizer, and evaporation (drift tube) temperature. Since the ELSD response is critical for the evaluation of low level impurities, the influence of the parameters is shortly discussed using Asp and mexilitine HCl test solutions:

1. The detector response increases as a function of the percentage of organic modifier in the eluent. The relation between the content of organic modifier in the eluent and the detector response of a 0.02 mg/mL solution of mexiletine HCl is presented in Fig. 1. Similar results were reported for triglycerides in different organic solvents measured by direct injection [15], for hydrocortisone acetate [16], and for 5-fluorocytosine [17], all using acetonitrile/water eluents. This effect can be explained by an increase of the transport efficiency of the nebulizer with increasing amounts of organic modifier in the eluent. This means that whilst the average particle size remains constant a greater number of particles reach the detection chamber, resulting in an increased detector signal [15–20].

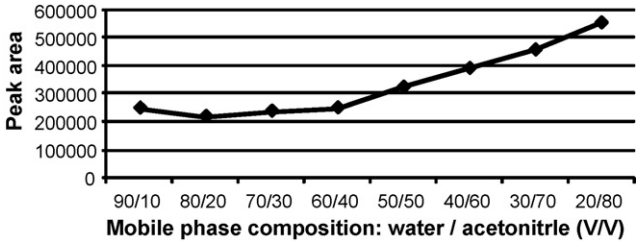

Fig. 1. Relation between the eluent composition and the detector response. *Conditions*: 0.02 mg/mL solution of mexiletine HCl in water/methanol (50/50, v/v); injection volume: 40 μL; eluent flow-rate: 0.5 mL/min; eluent composition modified in 10% (v/v) steps from water/acetonitrile (90/10, v/v) to water/acetonitrile (20/80, v/v); ELSD scavenger gas flow-rate: 1.0 SLM; nebulization and evaporation temperature: 50 °C.

2. The peak area decreases with increasing eluent flow-rate. This correlation is demonstrated for two examples (see Fig. 2): (a) 0.02 mg/mL solution of alfadex using a mixture of water/acetonitrile (90/10, v/v) as eluent, and (b) a 0.02 mg/mL solution of Asp using a mixture of water/methanol (80/20, v/v) as. All measurements were performed at the same scavenger gas flow-rate of 1.0 SLM.

Explanations for reduction of signal intensity are given in the literature: (a) at higher eluent flow-rates larger droplets are formed and the loss of the larger particles by impaction on the surfaces of the drift tube or spray chamber may be responsible [21], and (b) incomplete solvent vaporization occurring at higher eluent flow-rates [22,23]. However, it is difficult to give the main reason because all effects more or less contribute to the decrease of the peak area.

3. The ELSD used in our experiments allows adjustment of the scavenger gas flow-rate in the nebulizer compartment of the detector. The detector signal was found to decrease with increasing scavenger gas flow-rate (see Fig. 3) which is in accordance with findings obtained in direct infusion studies on olive oil [21], polystyrene [24] and glycerol [25]. The increase in the gas flow-rate causes a decrease in signal response due to the reduced particle size and the reduction in residence time of the particles within the optics. This finding seems to be in contradiction with the above statement that the sensitivity is reduced when larger droplets are formed. However, in both cases this finding is referred to the signal intensity at optimum average conditions. The reason for signal reduction at high eluent flow-rates is different from the one causing signal reduction at high gas flow-rates.

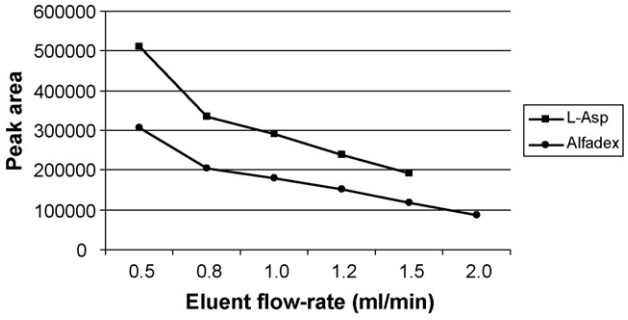

Fig. 2. Relation between the detector response of L-aspartic acid and alfadex at increasing eluent flow-rates. *Conditions*: 0.02 mg/mL solutions of Asp and alfadex both dissolved in water. ELSD settings as described in experiment 2.3.1. Eluent composition for Asp: water/methanol (80/20, v/v); for alfadex: water/acetonitrile (90/10, v/v). The analysis for water/methanol: injection volume of 40 μL at eluent flow-rates of 0.5 mL/min, 0.7 mL/min, 0.8 mL/min, 1.0 mL/min, 1.2 mL/min and 1.5 mL/min; for water/acetonitrile a flow rate of 2.0 mL/min.

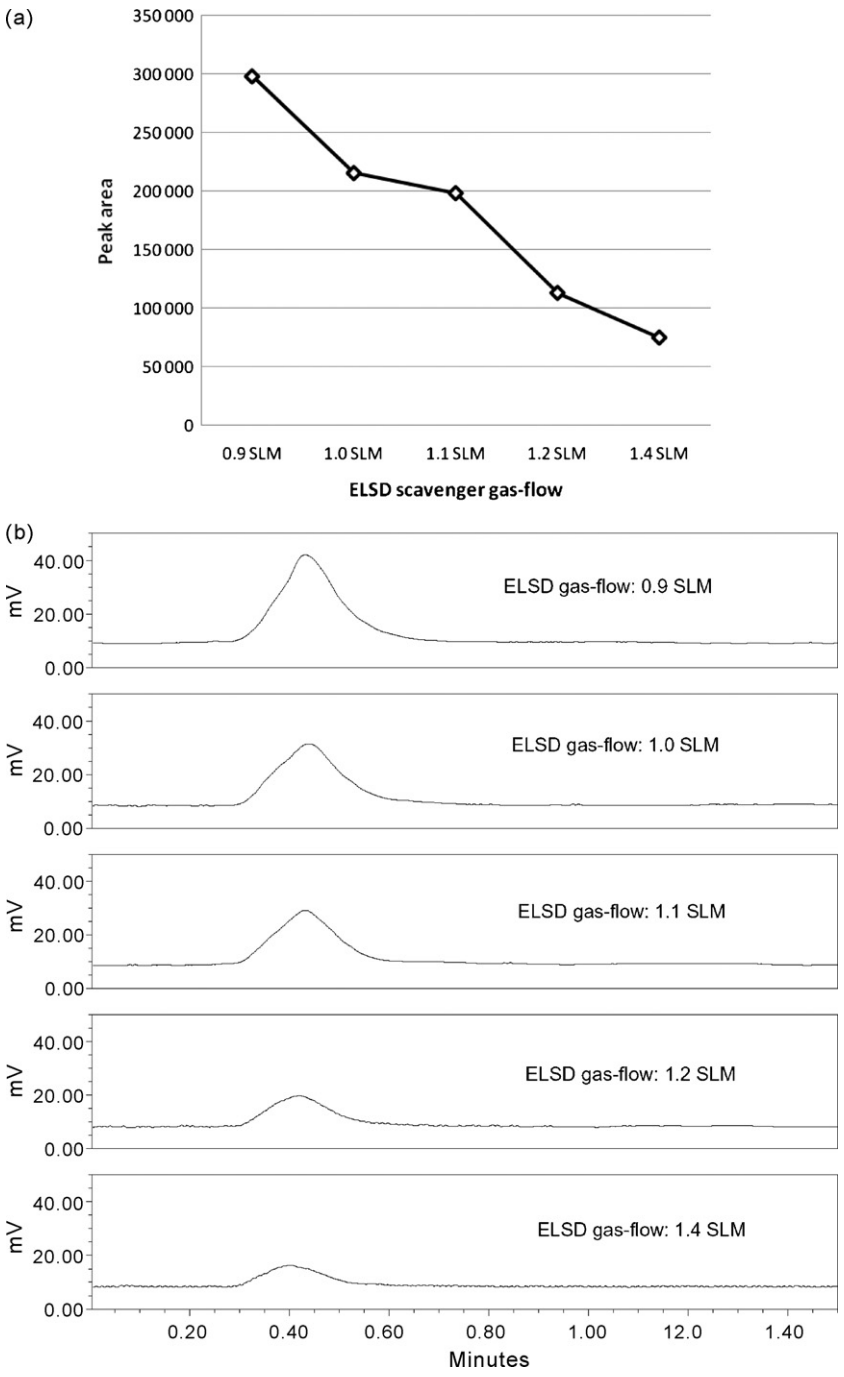

Fig. 3. (a) Relation between the detector response at different scavenger gas flow-rates. *Conditions*: Test solution: 0.02 mg/mL Asp in water; injection volume: 40 μL; eluent flow-rate: 0.5 mL/min; eluent: water/methanol (80/20, v/v); ELSD scavenger gas flow-rates of 0.9 SLM, 1.0 SLM, 1.1 SLM, 1.2 SLM and 1.4 SLM. (b) Decrease of the peak area of Asp at increasing scavenger gas flow-rates. Conditions as given for Fig. 4a. Signal-to-noise ratio decreases from about 200 at 0.9 SLM to about 25 at 1.4 SLM.

As stated above the cause in the first case is a loss of analyte particles in the drift tube whilst in the latter case possible reasons are a change in the light scattering process due to reduction of the average particle size and a reduced detector residence time.

4. Regarding the influence of the drift tube temperature, the highest signal intensity was found at 60 °C (see Fig. 4). At temperatures below 60 °C solvent evaporation is still incomplete and therefore the ELSD signal as a function of the particle size of the evaporated solid particles is low. At a temperature of about 60 °C the drying conditions seem to be optimal and the highest detector output is obtained. At a temperature higher than 60 °C the signal begins to decrease again. Since aspartic acid is not volatile, evaporation of the solute cannot be the reason.

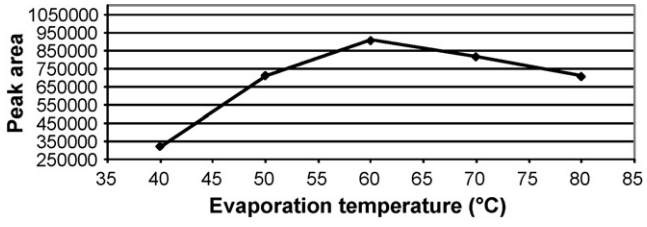

Fig. 4. Relation between the detector response of Asp at different evaporation (drift tube) temperature. Test solution: 0.02 mg/mL Asp in water; eluent: water/methanol (80/20, v/v); eluent flow-rate: 0.5 mL/min; injection volume: 40 μL; ELSD scavenger gas flow-rate: 1.0 SLM; nebulizer temperature: 30 °C; evaporation temperature increased from 40 °C to 80 °C in 10 °C steps.

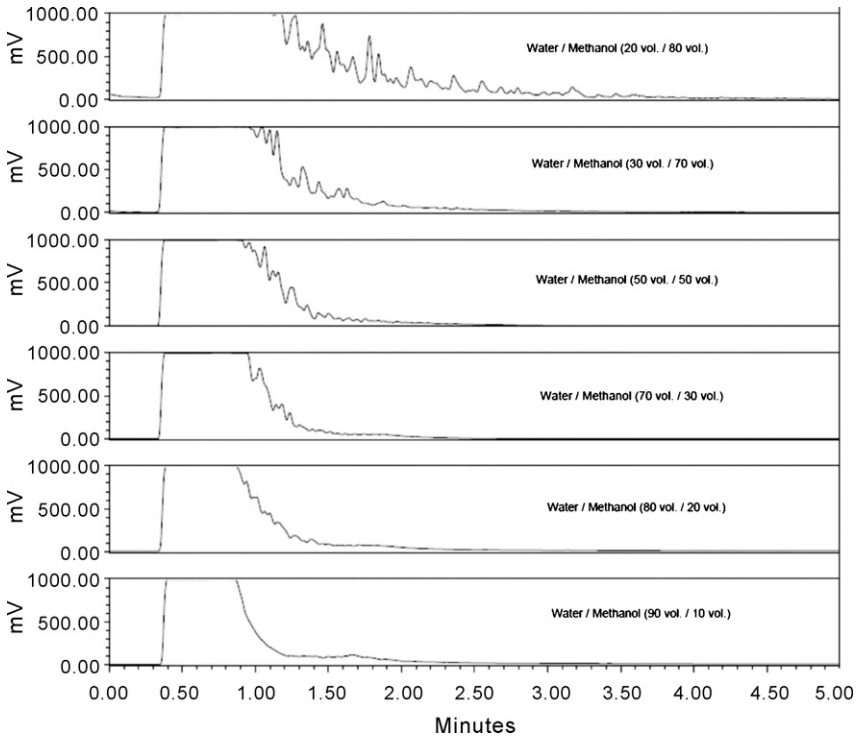

Fig. 5. Effect of eluent composition, i.e. water/methanol ratio at 40 mg/mL mexiletine HCl. *Conditions*: injection volume: 40 μL, eluent flow-rate: 0.5 mL/min; eluent composition modified in 10% (v/v) steps from water/acetonitrile (90/10, v/v) to water/acetonitrile (20/80, v/v); ELSD scavenger gas flow-rate: 1.0 SLM; nebulization and evaporation temperature: 50 °C.

A possible explanation for this response behaviour is that at high drift tube temperatures too rigorous solvent volatilization causes non-uniform particle sizing or inhibits crystal formation, adversely affecting the light scattering process [25].

3.2. Influence of the eluent composition, eluent flow and ELSD settings on the appearance of spike peaks

As shown above, the detector sensitivity may vary substantially depending on the corresponding eluent composition, eluent flow and ELSD settings. Therefore, a 0.05% dilution of the concentrated test solution has to deliver still a peak above the limit of quantification. This was on the one hand to ensure that appearance/disappearance of spikes is not simply a function of the method sensitivity and on the other hand to demonstrate that the selected sample concentration would be appropriate to control impurities in a pharmaceutical substance according to Q3A(R) [1]. In the following paragraphs the influence of different eluent composition, eluent flow and ELSD settings on the appearance of spike peaks is reported.

3.2.1. Influence of the eluent composition

Injecting a 40 mg/mL solution of Asp (see 2.3.1), a strong increase of spike peaks was found with increasing amounts of the organic modifier for both methanol and acetonitrile (see Fig. 5). Since the experiments were performed as FIA (without analytical column), these peaks cannot be due to separated impurities, but must be related to the substance itself.

Asp is known to show low solubility in organic solvents. To ensure that the spikes are not simply due a precipitation in the presence of high amounts of organic modifier, the tests were repeated using a 40 mg/mL solution of mexiletine HCl – a substance showing good solubility in water and in the organic modifiers used. However, the results were the same and spike peaks increased with increasing amounts of organic modifier.

The experiment was performed at a relatively low eluent flow-rate of 0.5 mL/min and a scavenger gas flow-rate of 1.0 SLM. It is assumed that under these conditions the appearance of spike peaks is due to improper nebulization which results in the formation of large droplets. These droplets may not be fully evaporate in the drift tube and result in the occurrence of spike peaks in the detector [21,26]. At increased amounts of organic modifier the eluent evaporation is enhanced and less large droplets are lost due to impaction on the drift tube walls. Since more of the larger droplets reach the detector, spike peaks are much more pronounced at high concentrations of organic modifier than in highly aqueous eluents.

3.2.2. Influence of the eluent flow-rate

Employing the experimental conditions given under 2.3.2 40 mg/mL test solutions of Asp, alfadex and mexiletine hydrochloride, respectively, were injected at an eluent flow-rate of 0.5 mL/min using either water/methanol or water/acetonitrile as eluent. Whilst no spikes were found when injecting either water as a blank or a 0.05% dilution of the test solution, several spikes were observed on the tail of the main peaks in each test solution.

To examine the influence of the eluent flow-rate, injections at increasing flow-rates, were conducted: 0.5 mL/min, 0.7 mL/min, 0.8 mL/min, 1.0 mL/min, 1.2 mL/min and 1.5 mL/min for water/methanol. For water/acetonitrile as eluent a flow-rate of 2.0 mL/min was tested.

It was found that at eluent compositions of water/methanol (80/20, v/v) and water/acetonitrile (90/10, v/v) the spikes significantly decreased or even disappeared when the eluent flow-rate was increased to 0.7 mL/min and higher. At flow rates of 1.0 mL/min and 1.2 mL/min, in none of the injections a spike peak was found. Using in turn, an eluent of a high organic concentration, e.g. water/methanol (20/80, v/v), spike peaks could also be significantly reduced at increased eluent flow-rates. However, in this case a flow-rate of at least 1.0 mL/min was required (see Fig. 6).

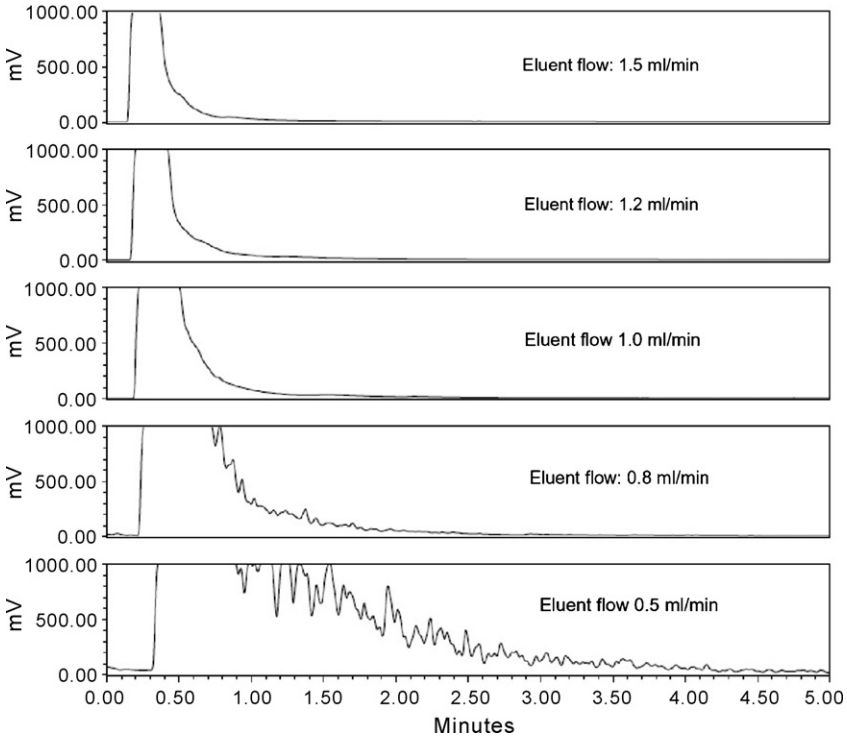

Fig. 6. Decrease of spike peaks for 40 mg/mL mexiletine HCl in water/methanol (20/80, v/v) at increasing eluent flow-rates. *Conditions*: ELSD settings as described in 2.3.1. Injection volume: 40 μL; eluent flow-rates: 0.5 mL/min, 0.7 mL/min, 0.8 mL/min, 1.0 mL/min, 1.2 mL/min and 1.5 mL/min.

It is assumed that at the selected scavenger gas flow-rate of 1.0 SLM and low eluent flow-rates the nebulizer is not working properly to form a suitable aerosol. Huge droplets may occasionally be formed resulting in spikes in the detector response [25].

3.2.3. Influence of the ELSD scavenger gas flow-rate

Test solutions containing 40 mg/mL of Asp and alfadex were injected using a mixture of water/methanol (80/20, v/v) as an eluent. The solutions were analysed at the different combinations of

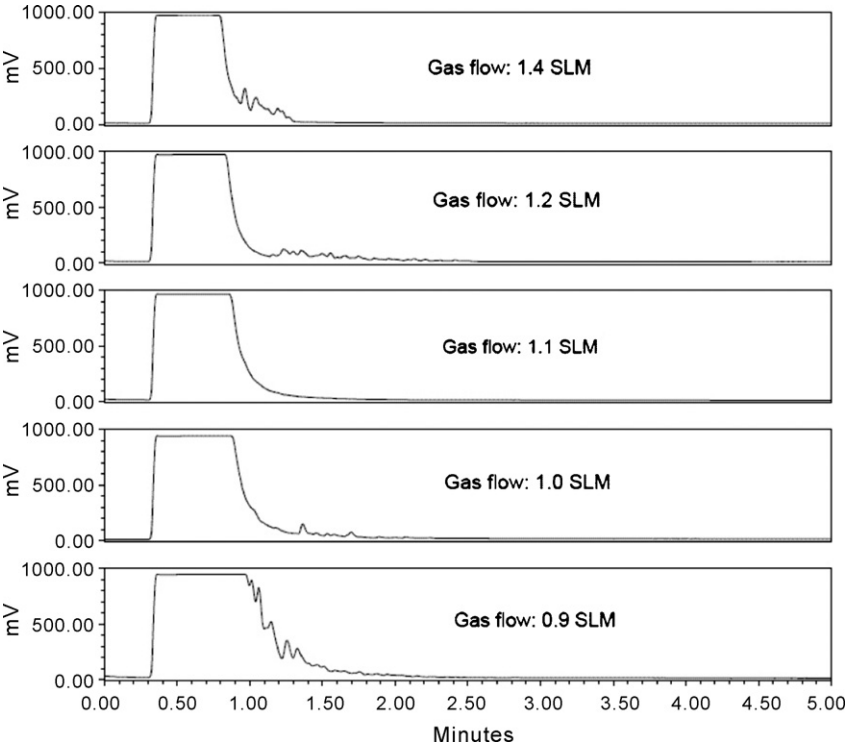

Fig. 7. Appearance of spike peaks at different scavenger gas flow-rates for Asp using an eluent with low amounts of organic modifier. *Conditions*: Test solution: 40 mg/mL of Asp; injection volume: 40 μL; eluent: water/methanol (80/20, v/v); eluent flow-rate: 0.5 mL/min; ELSD nebulization and evaporation temperature: 50 °C; ESLD scavenger gas flow-rates: 0.9 SLM, 1.0 SLM, 1.1 SLM, 1.2 SLM and 1.4 SLM.

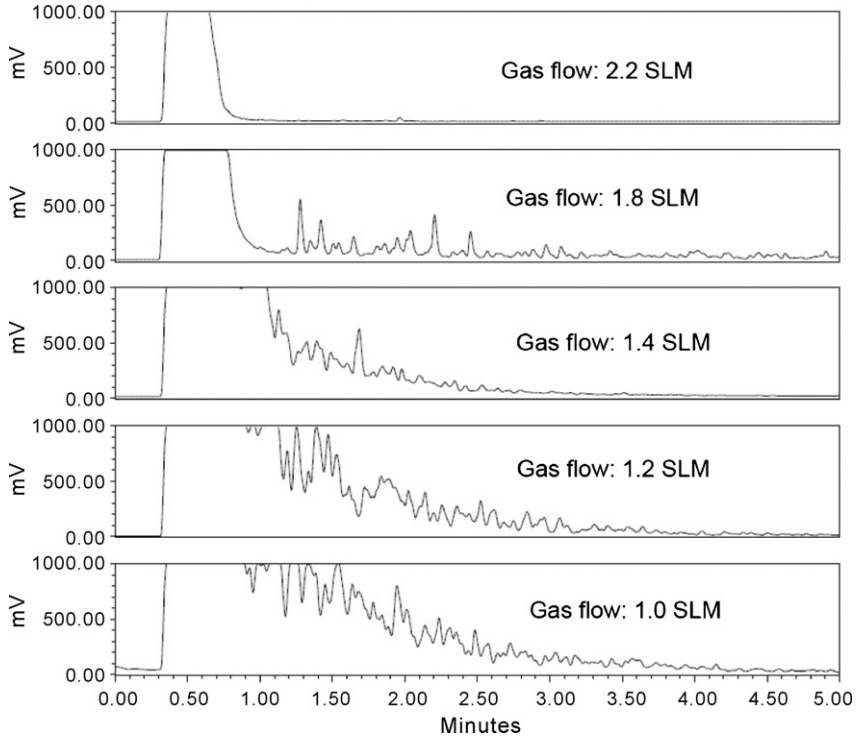

Fig. 8. Appearance of spike peaks at different scavenger gas flow-rates for mexiletine HCl using an eluent with high amounts of organic modifier. *Conditions*: Test solution: 40 mg/mL of mexiletine hydrochloride; injection volume: 40 μL; eluent: water/methanol (20/80, v/v); eluent flow-rate: 0.5 mL/min; ELSD nebulization and evaporation temperature: 50 °C; ESLD scavenger gas flow-rates: 1.0 SLM, 1.2 SLM, 1.4 SLM, 1.8 SLM and 2.2 SLM.

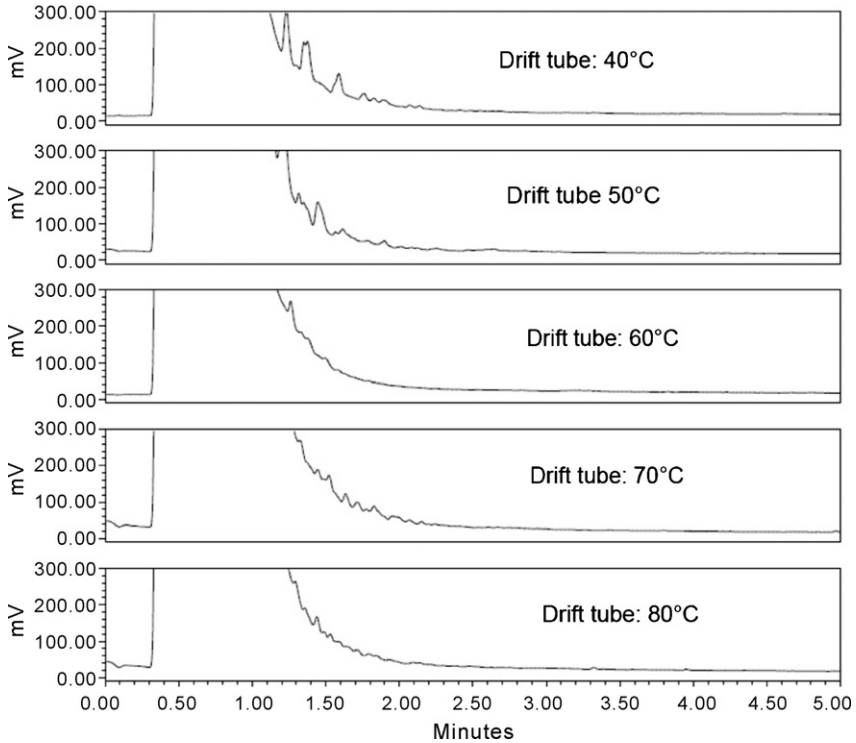

Fig. 9. Appearance of spikes at drift tube temperatures from 40 °C to 80 °C. *Conditions*: Test solution: 40 mg/mL Asp; injection volume: 40 μL; eluent: water/methanol (80/20, v/v); eluent flow-rate: 0.5 mL/min; ELSD scavenger gas flow-rate: 1.0 SLM; nebulizer temperature: 30 °C; evaporation temperature: increased from 40 °C to 80 °C in 10 °C steps.

eluent flow-rate and ELSD flow-rate (see 2.2.3). Spikes eluting after the principal peak were systematically found at scavenger gas flow-rates of 0.9 SLM and 1.0 SLM. However, at a scavenger gas flow-rate of 1.1 SLM no spikes were detected in three successive injections of the Asp and alfadex test solutions. A further increase to 1.2 SLM led to spike peaks in one out of three injections of Asp and to a few very small spikes in two out of three injections of the alfadex solution. At a gas flow-rate of 1.4 SLM spikes were found in the three injections of Asp. However, no spikes were found when alfadex was injected. Moreover a spike peak was found, in one out of three injections of the Asp test solution at a combination of 1.2 mL/min of eluent flow-rate and 1.4 SLM ELSD gas flow-rate. Fig. 7 shows the appearance of spike peaks for Asp at different ELSD gas flow-rates for an eluent flow-rate of 0.5 mL/min.

For eluents containing high amounts of organic modifier, the reduction of spike peaks by increasing the scavenger gas flow-rate was found to be much more difficult. Using a mixture of water/methanol (20/80, v/v) as eluent, a significant reduction of spike peaks for a 40 mg/mL solution of mexiletine HCl was only obtained when a scavenger gas flow-rate of 2.2 SLM was employed (see Fig. 8). However, as pointed out above, this goes together with a strong reduction in the sensitivity: In the 0.05% dilution of the mexiletine HCl test solution peaks were no longer detectable. The reason for the spike peaks might be the same as discussed under 3.2.1.; i.e. under the selected conditions large droplets are formed due to improper nebulization and as a consequence the evaporation of the solvent in the drift tube is incomplete, which results finally in the detection of spike peaks on the tail of the main substance signal. Since at increased amounts of organic modifier in the eluent more large droplets reach the detector, this phenomenon is much more pronounced compared to highly aqueous eluents.

3.2.4. Influence of the nebulizer and evaporation (drift tube) temperature

The detector used in this study allows independent variation of the nebulizer temperature and the drift tube (evaporation) temperature. In the two sets of experiments described in 2.3.4, the influence of a modification of the temperature on the appearance of spike peaks was investigated. In the first experimental series the nebulizer and the drift tube temperature were modified, in the second set, the nebulizer was kept at a constant (low) temperature and only the drift tube temperature was modified. However, regarding the influence on the signal intensity on the appearance of spike peaks, no differences were found. Due to the high percentage of water in the eluent, excessive noise was measured at an evaporation temperature of 30 °C and no signal could be measured. Therefore, the measurements could only be started at 40 °C.

Regarding the appearance of spike peaks when injecting concentrated solutions of Asp (c = 40 mg/mL) under the conditions described under 2.3.4, only a limited influence of the drift tube temperature was noticeable. Spike peaks were slightly reduced up to a temperature of 60 °C, but did not completely disappear even when the evaporation temperature was further increased to 80 °C (Fig. 9).

The slight improvement regarding the reduction of spike peaks at increased temperatures may be due to an improved drying of the droplets. However, increasing the evaporation temperature is not an option for analysis involving volatile substances or – as in the control of impurities in pharmaceutical substances – substances of unknown structure. In these cases, the lowest possible evaporation or drift tube temperature ensuring evaporation the eluent have to be chosen.

4. Conclusion

HPLC coupled to an ELSD may be a suitable alternative when it comes to the analysis of substances lacking a chromophor for UV detection. For methods controlling impurities in drug substances, the low limit of detection is a key factor. Consequently, very often highly concentrated analyte solutions are employed in order to quantify impurities at a level of 0.05% or even 0.03% next to high amounts of the analyte.

Eluent composition and flow as well as the selected ELSD settings have a significant influence on method sensitivity and may therefore help to increase the quantification limit of a given method. Unfortunately, most parameters increasing method sensitivity also foster the appearance of spike peaks eluting on the tail of the principal peak. Summarizing the results presented here, it can be concluded, that the appearance of spike peaks is particularly a problem when working at low eluent and scavenger gas flow-rates and with high amounts of organic modifier in the eluent. Especially when working with high percentages of organic modifier, an increase of the eluent flow-rate was found to be more effective to reduce spike peaks than an increase in the scavenger gas flow-rate. For the detector used in this study it can be concluded that even for eluents containing high amounts of organic modifier the application of eluent flow-rates at or above 1.0 mL/min together with scavenger gas flow-rates of minimum 1.1 SLM were suitable measures to avoid the appearance of spike peaks even at high solute concentrations.

Taken together, the development of a robust LC–ELSD method for the control of impurities in drug substances both LC and ELSD parameters must be carefully chosen to find the best compromise between sensitivity and avoidance of spike peaks.

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