Journal of Chromatography A, xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Full length article

Flexible nano- and microliter injections on a single liquid chromatography–mass spectrometry system: Minimizing sample preparation and maximizing linear dynamic range

Arnaud Lubin[a], Sheng Sheng[b], Deirdre Cabooter[c], Patrick Augustijns[c], Filip Cuyckens[a,*]

[a] *Discovery Sciences, Janssen Research and Development, Turnhoutseweg 30, B-2340 Beerse, Belgium*
[b] *Faculté des Sciences d'Orsay, Paris Sud University, 15 rue Georges Clemenceau, 91405 Orsay, France*
[c] *Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, O&N II Herestraat 49, 3001 Leuven, Belgium*

ARTICLE INFO

Article history:
Received 11 May 2017
Received in revised form
22 September 2017
Accepted 22 September 2017
Available online xxx

ABSTRACT

Lack of knowledge on the expected concentration range or insufficient linear dynamic range of the analytical method applied are common challenges for the analytical scientist. Samples that are above the upper limit of quantification are typically diluted and reanalyzed. The analysis of undiluted highly concentrated samples can cause contamination of the system, while the dilution step is time consuming and as the case for any sample preparation step, also potentially leads to precipitation, adsorption or degradation of the analytes.

© 2017 Elsevier B.V. All rights reserved.

An injection set-up composed of 3 valves that allows the injection of two distinct volumes with a 200-fold difference (2 μL and 10 nL) on a single LC/MS system is introduced. As a proof of concept, the set-up was applied to the analysis of haloperidol in human plasma.

By applying two different injection volumes, it was possible to double the linear dynamic range of a liquid chromatography – triple quadrupole mass spectrometry instrument. For the analysis of haloperidol in plasma, the linear dynamic range could be increased from 25–5,000 ng/mL to 25–1,000,000 ng/mL. Both 10 nL and 2 μL injection modes showed very similar accuracy and precision. Additionally, more than 1,000 direct injections of untreated plasma were successfully carried out using a nanoliter valve with no measurable effect on LC performance (retention time, peak width, column pressure and background signal).

1. Introduction

A common challenge faced by analytical chemists is the analysis of samples for which the expected concentration range is unknown or the linear dynamic range of the analytical method is insufficient. Samples with concentrations above the upper limit of quantification are typically diluted and re-analyzed. Extra sample preparation is thus required and when done manually, it's a time-consuming task and a common source of error [1,2]. Moreover, as for any sample manipulation, a dilution step introduces a potential risk for degradation or loss (precipitation or adsorption) of the analyte(s) of interest. A simple alternative can be the reinjection of samples exceeding the upper limit of quantification as well as calibration and control samples with a lower injection volume, hence increasing the linear dynamic range of the analytical method. However, the applicable range of traditional ultra-high pressure liquid chromatography (UHPLC) injection systems is often too limited by column loadability and by the minimum injection volume that can be injected with sufficient repeatability (usually 10–25% of the injection loop volume). By reducing the minimum injection volume to a few nanoliters, the linear dynamic range can be extended significantly. Several injection techniques from the micro-LC and nano-LC environment are available for nanoliter injections. However, the flexibility to also inject microliter volumes is always lost [3–5].

The possibility to perform nanoliter injections on standard UHPLC systems also opens up new possibilities. Conventionally, protein precipitation, liquid/liquid extraction and solid phase extraction are used as sample preparation techniques for the analysis of protein-rich samples [6–8]. However, instability of drugs, prodrugs and their metabolites is a common issue in bioanalysis, and in addition to being time consuming, each step in these sam-

* Corresponding author at: Janssen Research and Development, Turnhoutseweg 30, B-2340, Beerse, Belgium.
E-mail addresses: alubin@its.jnj.com (A. Lubin), ssheng.upsud@gmail.com (S. Sheng), deirdre.cabooter@kuleuven.be (D. Cabooter), patrick.augustijns@kuleuven.be (P. Augustijns), FCUYCKEN@its.jnj.com (F. Cuyckens).

https://doi.org/10.1016/j.chroma.2017.09.053
0021-9673/© 2017 Elsevier B.V. All rights reserved.

ple preparation techniques can cause a degradation or a loss of analyte resulting in an underestimation of the concentration [8,9]. Therefore, efforts have been made to perform direct injection of plasma [10,11]. Such techniques include micellar liquid chromatography, restricted access materials and online solid phase extraction [12–15]. Direct injection of plasma with reverse phase liquid chromatography is not routinely performed since the proteins present in plasma are known to irreversibly adsorb on the stationary phase increasing the back pressure of the column and degrading the LC performance over time [10,12]. However, using a nanoliter injection volume it is possible to perform a large number of injections without observing any degradation of the LC performance. With the ever-increasing sensitivity of MS instrumentation, a growing interest and applicability of this approach is to be expected.

In this article, we present an injection set-up composed of three valves that allow the injection of two volumes with a 200-fold difference (2 μL and 10 nL). The developed set-up is first described in detail before being applied for the analysis of haloperidol in human plasma as a proof of concept. In addition, the benefit of having nanoliter injection volumes available was used for the direct injection of 1,000+ plasma samples on a reverse phase column. Applications of the set-up, its limitations and further developments are also discussed.

2. Materials and methods

2.1. Chemicals and reagents

Methanol and formic acid of analytical grade were purchased from Merck (Darmstadt, Germany). Ammonium formate and dimethyl sulfoxide, also of analytical grade, were purchased from VWR (Leuven, Belgium). Haloperidol was synthesized in-house at Janssen Pharmaceutica (Beerse, Belgium). Haloperidol-D4 was purchased from Toronto Research Chemicals (Downsview, ON, Canada). Atenolol, lincomycin hydrochloride, terfenadine and buspirone were purchased from Sigma (Steinheim, Germany). Ultrapure water (H_2O) was produced with a MilliQ system (Millipore, Billerica, MA, USA). Human plasma (EDTA) was obtained from Bioreclamation Inc. (Hicksville, NY, USA).

2.2. 10 nL–2 μL injection set-up

Injections were carried out on an Acquity UPLC system (Waters, Milford, MA, USA) coupled to an API 4000 triple quadrupole mass spectrometer (AB Sciex, Framingham, MA, USA). Next to the standard injection system, two valves were added: a 4 ports/2 positions, 1/16" fitting, 0.15 mm bore diameter valve with a 10 nL internal sample injector and a 6 ports/2 positions, 1/16" fitting, 0.25 mm bore diameter valve (both from VICI AG International, Schenkon, Switzerland), hereinafter referred as nanoliter valve and selection valve, respectively.

The injection system was set up to allow two injection modes: full loop (injection volume defined by loop size e.g. 2 μL) and a fixed volume of 10 nL by the use of two additional valves. A 4 ports internal sample injector valve (nanoliter valve) was connected to the waste line of the UHPLC valve and to a 6 ports valve (selection valve) which was also connected to the column and pump flow port of the UHPLC valve as shown in Fig. 1. Two 2 μL loops were used as connections between the selection valve and the UHPLC valve. A 5 μL loop was used to connect the nanoliter valve and the selection valve whereas ETFE (1/16" O.D. × 0.25 mm I.D.) tubing was used as an extension of the sample line between the UHPLC and the nanoliter valve. A 2 μL loop was used for the full loop injection mode. All the valves were placed as close as possible to each other in order to minimize the dead volume. The actuators of the valves were connected to the sample manager of the UHPLC system and allowed to switch events by digital control.

Before starting any injection, the selection valve was switched in a position that allowed either a full loop (2 μL) or a 10 nL injection. The loading was the same for both injection modes; a full loop injection was used to fill-up the loop and the nanoliter valve (Fig. 1a). Depending on the position of the selection valve, either 2 μL was injected (Fig. 1b) or 10 nL if the nanoliter valve was switched (Fig. 1c). As one can see in Fig. 1c, the 10 nL injection is possible because the pathway between the two connections is calibrated and is used as internal sample injector. In fact, the nanoliter valve has a rotation of 90°, instead of the 60° rotation used on regular 6 ports valves. As a result, it is possible to transfer the content of the valve's pathway from the sample line directly into the mobile phase flow. A video available as online Supplementary material shows in detail each step of the two injection modes.

2.3. LC–MS conditions

2.3.1. Analysis of haloperidol

Separations were performed on an Acquity UPLC BEH C18 2.1 × 50 mm (1.7 μm I.D. particles) column (Waters, Milford, MA, USA) held at 60 °C during all experiments. 10 mM ammonium formate adjusted to pH 4 with formic acid (eluent A) and methanol (eluent B) were used as eluent with a gradient from 95% A to 100% B in 4 min at 350 μL/min. The final mobile phase composition was held constant during 1 min, brought back to 95% A in 0.1 min and kept constant for 1.4 min to re-equilibrate the column. For both injection modes, the overfill factor was set to 13, corresponding to 26 μL of sample consumed per injection. 2.4 mL of weak wash (Eluent A/Eluent B, 50/50, v/v) and 1.4 mL of strong wash (methanol/dimethyl sulfoxide, 50/50, v/v) were used per injection. Wash volumes larger than the default recommended volumes were used in order to clean the sample line up to the nanoliter valve.

For the analysis of haloperidol in plasma, an API4000 triple quadrupole mass spectrometer (SCIEX, Streetville, ON, Canada) equipped with an ESI source was operated in positive ion mode using the following selected reaction monitoring (SRM) transitions: 376 > 123 for haloperidol and 380 > 123 for haloperidol-D4 (internal standard). Details about the source conditions for all experiments can be found Table S-1 in the Supplementary information.

2.3.2. 1,000+ direct plasma injections

To avoid any precipitation of proteins from plasma samples on the column, the gradient was set to start at 100% aqueous phase. Therefore, an Acquity UPLC HSS T3 2.1 × 50 mm (1.8 μm) column (Waters) compatible with 100% aqueous mobile phase was used. The column was held at 45 °C during all experiments. 10 mM ammonium formate adjusted to pH 4 with formic acid (eluent A) and methanol (eluent B) were used as eluent with a gradient from 100% A to 100% B in 1 min at 400 μL/min. The final mobile phase composition was held constant during 0.5 min, brought back to 100% A in 0.05 min and kept constant for 0.2 min to re-equilibrate the column. For this experiment, the overfill factor was set to 10 (20 μL of sample consumed). 2.0 mL of weak wash (water) and 0.5 mL of strong wash (methanol/dimethyl sulfoxide, 50/50, v/v) were used per injection.

The API4000 MS system was operated in positive ion mode with the following SRM transitions: 376 > 165 for haloperidol, 267.1 > 145.2 for atenolol, 386.2 > 122.1 for buspirone, 472.3 > 436.2 for terfenadine and 407.2 > 126.1 for lincomycin.

Please cite this article in press as: A. Lubin, et al., Flexible nano- and microliter injections on a single liquid chromatography–mass spectrometry system: Minimizing sample preparation and maximizing linear dynamic range, J. Chromatogr. A (2017), https://doi.org/10.1016/j.chroma.2017.09.053

Download English Version:

https://daneshyari.com/en/article/7609641

Download Persian Version:

https://daneshyari.com/article/7609641

Daneshyari.com